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Dialog level 05.24.00D

? b 411;set files biotech

30jan09 17:30:47 User219511 Session D756.2

\$0.00 0.117 DialUnits File410

\$0.00 Estimated cost File410

\$0.06 TELNET

\$0.06 Estimated cost this search

\$0.62 Estimated total session cost 0.273 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

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? s (bmp4 or bmp-4 or rbmp4 or rbmp-4 or rhbmp4 or rhbmp-4) and immobili? and (graft? or scaffold?)

Your SELECT statement is:

s (bmp4 or bmp-4 or rbmp4 or rbmp-4 or rhbmp4 or rhbmp-4) and immobili?  
and (graft? or scaffold?)

Items File

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2 357: Derwent Biotech Res.\_1982-2008/Nov W5

1 file has one or more items; file list includes 27 files.

? save temp; b 357,exs

Temp SearchSave "TG646450105" stored

30jan09 17:32:01 User219511 Session D756.3

\$2.47 0.841 DialUnits File411

\$2.47 Estimated cost File411

\$0.53 TELNET

\$3.00 Estimated cost this search

\$3.62 Estimated total session cost 1.113 DialUnits

File 357:Derwent Biotech Res.\_1982-2008/Nov W5

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Set Items Description

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Executing TG646450105

70 BMP4

8 BMP-4

0 RBMP4

1 RBMP-4

0 RHBMP4

0 RHBMP-4

32803 IMMOBILI?

4806 GRAFT?

1818 SCAFFOLD?

S1 2 (BMP4 OR BMP-4 OR RBMP4 OR RBMP-4 OR RHBMP4 OR RHBMP-4)  
AND IMMOBILI? AND (GRAFT? OR SCAFFOLD?)

? t s1/7/1-2

1/7/1

DIALOG(R)File 357:Derwent Biotech Res.

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0316052 DBR Accession No.: 2003-17192 PATENT

Transferring nucleic acid into cells associated with fluid space by  
contacting wound site situated in tissue associated with fluid space,  
with composition comprising nucleic acid and biocompatible matrix -  
gene transfer expression in cell for use in disease therapy and gene  
therapy

AUTHOR: SOSNOWSKI B A; PIERCE G

PATENT ASSIGNEE: SELECTIVE GENETICS INC 2003

PATENT NUMBER: WO 200329429 PATENT DATE: 20030410 WPI ACCESSION NO.:  
2003-430202 (200340)

PRIORITY APPLIC. NO.: US 327513 APPLIC. DATE: 20011003

NATIONAL APPLIC. NO.: WO 2002US31546 APPLIC. DATE: 20021002

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Transferring (M1) a nucleic acid molecule into cells associated with a fluid space, involves contacting a wound site with a composition (I) comprising a nucleic acid molecule and a biocompatible matrix, the wound site being situated in a tissue associated with the fluid space. DETAILED DESCRIPTION - INDEPENDENT

CLAIMS are also included for: (1) stimulating (M2) gene expression in cartilage progenitor cells located within a cartilage progenitor tissue

site of an animal, involves contacting the tissue site with a composition comprising a chondrogenic gene and a biocompatible matrix; (2) stimulating (M3) cartilage repair or regeneration, by implanting at a cartilage defective site a matrix-gene composition comprising a chondrogenic gene and a biocompatible matrix; (3) treating (M4) arthritis, by implanting at a cartilage defective site a matrix-gene

(4) position comprising chondrogenic gene and a biocompatible matrix; (5) treating (M5) ischemic heart disease by implanting a matrix-gene composition comprising an angiogenic gene and a biocompatible matrix into an ischemic region; and (6) a composition comprising multiple genes associated with a multi-partitional biocompatible matrix.

BIOTECHNOLOGY - Preferred Method: The wound site is situated in a tissue e.g., cartilage, cardiac muscle or bone/cartilage interface, associated with the fluid space. The method involves contacting (I)

with a wound site which is a wound induced by injury or a disease state, or an iatrogenic wound. The contacting process involves bringing the nucleic acid molecule into contact with the biocompatible matrix to form a matrix-nucleic acid composition and bringing the matrix-nucleic acid composition into contact with the tissue site. The nucleic acid molecule is a DNA molecule complexed with anti-DNA antibodies, histone H1, a polycation or is a DNA molecule comprising a promoter operably linked to a sequence encoding a gene product. The DNA molecule encodes a therapeutic protein such as a growth factor chosen from transforming

growth factor (TGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF), bone morphogenic factor (BMP) or a cartilage-derived morphogenic protein (CDMP). Optionally, the therapeutic protein is a growth hormone or human parathyroid hormone (PTH). The therapeutic protein may be latent TGF-beta binding protein (LTBP), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), Factor VIII, Factor IX, erythropoietin (EPO), tissue plasminogen activator (TPA), leukemia inhibitory factor (LIF), parathyroid hormone-related peptide (PTHRP), activin, inhibin,

interleukin, macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), skeletal growth factor (SGF), chondromodulin, mono or polyclonal antibodies and its fragments, enzymes involved in production and/or processing of collagen, enzymes involved in production and/or processing of hyaluronic acid, transcription factors that trigger proliferation, differentiation, and morphogenic pathways, cell survival factors, or cell death factors.

Optionally, the nucleic acid molecule is an RNA molecule, antisense nucleic acid molecule, a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus. The biocompatible matrix is a biological matrix which comprises a polymer, and is chosen from collagen, purified proteins, purified peptides, polysaccharides (e.g. chitosan, alginate, dextran, hyaluronic acid and cellulose), and extracellular matrix compositions. Preferably, the biological matrix comprises type I collagen, type II collagen,

mineralized collagen or atelocollagen collagen. Optionally, the biocompatible matrix is a synthetic matrix which comprises a polymer chosen from polyethylene glycols or their derivatives, polyesters, polyethers, polyhydrides, polyalkylcyanoacrylates, polyacrylamides, polyorthoesters, polyphosphazenes, polyvinylacetates, block copolymers, polytetrafluoroethylene (PTFE), and polyurethanes. Optionally, the polymer comprises lactic acid or glycolic acid, or may be a copolymer

which comprises lactic acid and glycolic acid (PLGA). The biocompatible

matrix is biodegradable or non-biodegradable. The non-biodegradable matrix comprises a polymer such as poly(dimethylsiloxane) or poly(ethylene-vinyl acetate). The biocompatible matrix is a collagen, metal, hydroxyapatite, bioglass, aluminate, bioceramic materials, hyaluronic acid polymers, acrylic ester polymer, lactic acid polymer, glycolic acid polymer, lactic acid/glycolic acid polymer, purified proteins, purified peptides, and extracellular matrix compositions.

Preferred Method: In (M2), the contacting process involves bringing the chondrogenic gene with the biocompatible matrix to form a matrix-gene composition and bringing the matrix-gene composition into contact with the tissue site. The biocompatible matrix is a collagen preparation, hydroxyapatite matrix, a lactic acid polymer matrix or a fibrin matrix.

In (M3), the matrix comprises a first portion and a second portion. The first portion comprises a gene to stimulate cartilage growth and the second portion comprises a gene to stimulate bone growth.

**ACTIVITY - Vulnerary; Antiarthritic; Antinflammatory; Vasotropic.**

**MECHANISM OF ACTION** - Gene therapy. Influence of collagen-%immobilized% fibroblast growth factor (FGF) genes on muscle wound repair was examined using the rodent hind limb model. At day 14 following delivery of DNA(FGF2) formulated in a blend of 1% collagen and 1% gelatin, trichrome stains revealed that these matrices were well infiltrated by both mononuclear cells and elongated fibroblastoid cells. Many of these cells were organized around simple single-walled vessel, and may represent vascular precursors giving rise to neovasculature. The presence of erythrocytes with vessel lumens confirmed that these vessels were perfused. By day 21 post-treatment, in addition to microvasculature, well-organized muscular arterioles were also present. Skeletal muscle bundles were scattered throughout the collagen-gelatin matrix, which appeared to be reduced in volume over that seen at day 14. neither the residual matrix nor the surrounding tissue showed any signs of edema. Very similar observations were seen following the delivery of collagen-gelatin-DNA(FGF6) to muscle wounds, including the development of both micro- and macrovasculature. Delivery of the control transgene luciferase induced a much different response. Even at day 21, considerable collagen-gelatin matrix remained, and although a mononuclear cell infiltrate was present, blood-perfused vessels perfused were rare. infiltrating cells were organized into discrete areas, however the majority of these structures were not true vasculature in that they were not lined by a continuous endothelium and were not perfused with blood. Finally, delivery of FGF2 protein was seen to induce a limited angiogenic response comprised of small capillaries. Arteriogenesis similar to that induced by FGF2 or FGF6 gene delivery was not observed.

**USE** - (M1) is useful for transferring a nucleic acid molecule into cells associated with a fluid space. (M2) is useful for stimulating gene expression in cartilage progenitor cells located within a cartilage progenitor tissue site of an animal, where expression of the gene in the cell stimulates the cells to promote cartilage tissue repair or regeneration. The cartilage progenitor tissue site of an animal is a site of cartilage injury (a partial-thickness injury or a full-thickness injury), or is a cartilage cavity site, or is the result of surgery or the removal of cartilage tumor. The chondrogenic gene is in the form of plasmid DNA, a DNA insert within the genome of a recombinant adenovirus, a DNA insert within the genome of a recombinant adeno-associated virus (AAV) or a DNA insert within the genome of a recombinant retrovirus. The chondrogenic gene is parathyroid hormone (PTH) gene, bone morphogenic factor (BMP) gene, a cartilage-derived morphogenic protein (CDMP) gene, a growth factor gene, a growth factor receptor gene (e.g., IGF receptor gene or MBP receptor gene), where the growth factor gene is fibroblast growth factor (FGF) gene, insulin-like growth factor (IGF) gene, hepatocyte growth factor (HGF) gene, a gene in the transforming growth factor (TGF) family of genes, epidermal growth factor (EGF) gene, connective tissue growth factor (CTGF) gene, leukemia inhibitory factor (LIF) gene, parathyroid hormone-related peptide (PTHRP) gene, platelet-derived growth factor (PDGF) gene, skeletal growth factor (SGF) gene, BIP gene, MP52 gene, chondromodulin gene, preferably basic FGF gene, IGF-I or IGF-II gene, TGFalpha, TGFbeta1 or TGFbeta2, BMP2, BMP3, %%BMP4%%, BMP5, BMP6, BMP7, BMP8, BMP9, BMP10, BMP11, BMP12 or BMP13 gene. (M3) is useful for stimulating cartilage repair or regeneration. (M4) is useful for treating arthritis, where the

chondrogenic gene that is implanted is an IL-4 gene, or a gene that encodes either a ribozyme that cleaves mRNAs for an inflammation mediator, or an antisense nucleic acid that binds to mRNA for an inflammation mediator such as IL-1, IL-6, IL-8, TNF-alpha, granulocyte-macrophage colony stimulating factor (GM-CSF), a soluble receptor that binds to a mediator of inflammation, or an antibody or its fragment that binds to a mediator of inflammation. (M5) is useful for treating ischemic heart disease, where the angiogenic gene that is implanted is FGF gene, VEGF gene, TNF-alpha gene, HGF gene, or a PDGF gene (all claimed).

**ADMINISTRATION** - The gene-matrix composition is transferred directly to the site of a naturally occurring wound or an iatrogenic injury or the matrices may be surgically placed in a wound made in an organ. The matrices may also be implanted via %%grafting%%, injection, catheterization, laparoscopic surgical procedures, or arthroscopic surgery.

**ADVANTAGE** - Direct plasmid DNA transfer from a matrix to a mammalian repair cell, through stimulation of the wound healing process, has the following advantages: (a) each are capable of producing and purifying DNA constructs; (b) matrices can act as structural %%scaffolds%% that, in and of themselves, promote cell in growth and proliferation, thus facilitating the targeting of repair cells for gene transfer; (c) the introduction of a biocompatible matrix to tissues associated with a fluid space results in less damage to surrounding tissues during introduction; (d) the biocompatible matrix may be implanted through or across the fluid space without harming other tissue; (e) the method therefore, is a minimally invasive means of utilizing gene therapy to introduce therapeutic molecules to tissues associated with fluid spaces; (f) the proximity of a fluid space facilitates the migration of repair cells to the biocompatible matrix that is inserted into a tissue associated with a fluid space; and (g) the methods are efficient in introducing gene therapy products to target cells associated with a fluid space.(95 pages)

1/7/2

DIALOG(R)File 357:Derwent Biotech Res.

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0306427 DBR Accession No.: 2003-08212 PATENT

Regulating LRP5, LRP6 or HBM activity in a subject, useful for modulating lipid levels and/or bone mass, and for in treating bone mass disorders, e.g. osteoporosis, comprises administering a composition which modulates a Dkk activity - aptamer, antisense and reporter molecular for disease diagnosis and therapy

**AUTHOR:** ALLEN K; ANISOWICZ A; BHAT B M; DAMAGNEZ V; ROBINSON J A; YAWORSKY P J

**PATENT ASSIGNEE:** GENOME THERAPEUTICS CORP; WYETH 2002

**PATENT NUMBER:** WO 200202015 **PATENT DATE:** 20021121 **WPI ACCESSION NO.:** 2003-129219 (200312)

**PRIORITY APPLIC. NO.:** US 361293 **APPLIC. DATE:** 20020304

**NATIONAL APPLIC. NO.:** WO 2002US15982 **APPLIC. DATE:** 20020517

**LANGUAGE:** English

**ABSTRACT:** DERVENT ABSTRACT: NOVELTY - Regulating LRP5, LRP6 or HBM activity in a subject comprising administering a composition which modulates a Dkk activity, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) regulating Dkk-Wnt pathway activity in a subject; (2) modulating bone mass in a subject; (3) modulating lipid levels in a subject; (4) diagnosing low or high bone mass and/or high or low lipid levels in a subject; (5) screening for a compound which modulates the interaction of Dkk with LRP5, LRP6, HBM or a Dkk-binding fragment of LRP5, LRP6 or HBM; (6) screening a compound which modulates the interaction of Dkk with a Dkk interacting protein; (7) a composition comprising an LRP5, LRP6 or HBM activity-modulating compound, and a pharmaceutical carrier; (8) a pharmaceutical composition a compound which modulate Dkk and LRP5/LRP6/HBM interactions; (9) identifying binding partners for a Dkk protein or compounds which modulate Dkk and/or LRP5/LRP6/HBM interactions; (10) a nucleic acid encoding a Dkk interacting protein peptide aptamer comprising a nucleic acid encoding a %%scaffold%% protein in-frame with the activation domain of Gal4 or Lex A that is in frame with a nucleic acid that encodes a Dkk interacting protein amino acid

sequence; (11) a vector comprising the nucleic acid of (10); (12) detecting a modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide-binding pair that binds through extracellular interaction in their natural environment; (13) a transgenic animal where Dkk-1 is knocked out in a tissue-specific fashion; (14) identifying potential compounds which modulate Dkk activity; (15) a peptide aptamer comprising one of 22 13-32 residue amino acid sequences, given in the specification; (16) an antibody or antibody fragment which recognizes and binds to one or more of 18 13-17 residue amino acid sequences, given in the specification; (17) identifying Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway; (18) identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions; (19) identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway; (20) testing compounds that modulate Dkk-mediated activity in a mammal; (21) screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein; and (22) an antibody or antibody fragment which recognizes and binds to a sequence selected from 18 peptide sequences given in the specification.

**BIOTECHNOLOGY - Preferred Method:**

Dkk is Dkk-1, and Dkk activity is inhibited. The Dkk activity modulates bone mass and/or lipid levels, where bone mass is increased and/or lipid levels are decreased. Increase in bone mass is determined by a decrease in fracture rate, or by an increase in bone strength, bone density, trabecular connectivity, trabecular density, cortical density, bone diameter or inorganic bone content. The composition comprises one or more compounds selected from Dkk interacting proteins or its Dkk-binding fragment. The composition comprises an antisense, siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins. The composition may also comprise a mimetic of a Dkk peptide aptamer, a mimetic of a Dkk interacting protein peptide aptamer, a mimetic of a Dkk interacting protein peptide aptamer, or an LRP5 peptide aptamer. The composition inhibits or enhances Dkk binding to LRP5, LRP6 or HBM, or may also inhibit or enhance Dkk interacting protein or Dkk-binding fragment binding to Dkk. The peptide aptamer OST262 comprises a 154 residue amino acid sequence, given in the specification. The composition may alternatively comprise an LRP5 antibody or its immunologically active fragment. The subject is a vertebrate or an invertebrate, preferably a mammal selected from a canine, feline, ovine, primate, equine, porcine, caprine, camelid, avian, bovine and rodent, where the primate is preferably a human. Regulating Dkk-Wnt pathway activity in a subject comprises administering a composition which modulates Dkk activity, where Wnt is selected from Wnt1-Wnt19, preferably Wnt1, Wnt3, Wnt3a or Wnt10b. The composition which modulates Dkk activity or Dkk interaction with LRP5/LRP6/HBM is administered to modulate Wnt signaling. Modulating bone mass or lipid levels in a subject comprises administering a composition which modulates Dkk activity or Dkk interaction with LRP5, LRP6 or HBM, where bone mass is increased. Increase in bone mass is determined by a decrease in fracture rate, or by an increase in bone strength, bone density, bone mineral density, trabecular connectivity, trabecular density, cortical density, bone diameter or inorganic bone content. The subject has a bone mass disorder such as bone development disorder, bone fracture, age-related loss of bone, chondrodyostrophy, drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteoporosis, Paget's disease, osteoarthritis, or rickets. The composition is administered to modulate the amount of trabecular and/or cortical tissue. The lipid-modulated disorder is a cardiac condition, atherosclerosis, familial lipoprotein lipase deficiency, familial apoprotein CII deficiency, familial hypertriglyceridemia, multiple lipoprotein-type hyperlipidemia, elevated lipid levels due to dialysis and/or diabetes, or elevated lipid levels of unknown etiology. Diagnosing low or high bone mass and/or high or low lipid levels in a subject comprises examining expression of Dkk, LRP5, LRP6, HBM and/or HBM-like variant in the subject, and determining whether these are over- or under-expressed. Screening for a compound which modulates the interaction of Dkk with LRP5, LRP6, HBM or a Dkk-binding fragment of LRP5, LRP6 or HBM, comprises exposing Dkk and an LRP5, LRP6 and/or HBM binding fragment to a compound, and determining whether the compound

modulates Dkk interaction with the LRP5, LRP6 and/or HBM binding fragment, where modulation is determined by determining if the compound binds to Dkk or the LRP5, LRP6 and/or HBM binding fragment. The Dkk or an LRP-binding fragment is attached to a substrate. The compound comprises one or more Dkk interacting proteins or Dkk binding fragment, a Dkk peptide aptamer, a mimetic of a Dkk peptide aptamer, a Dkk interacting protein peptide aptamer, an LRP5 peptide aptamer, an LRP5 antibody, or a mimetic of a Dkk interacting protein peptide aptamer. Screening a compound which modulates the interaction of Dkk with a Dkk interacting protein comprises exposing a Dkk interacting protein or a Dkk-binding fragment to a compound, determining whether the compound binds to the Dkk interacting protein or Dkk-binding fragment, and further determining whether the compound modulates the interaction of Dkk interacting protein and Dkk. Identifying compounds, which modulate Dkk and/or LRP5/LRP6/HBM interactions, comprises creating an LRP5, LRP6 or HBM fluorescent fusion protein using a fluorescent tag, creating a Dkk fusion protein comprising a second fluorescent tag, adding a test compound, and assessing changes in the ratio of fluorescent tag emissions using fluorescence Resonance energy transfer (FRET) or bioluminescence resonance Energy Transfer (BRET) to determine whether the compound modulates Dkk and LRP5/LRP6/HBM interactions. The method may alternatively comprise %%%immobilizing%%% LRP5/LRP6/HBM to a solid surface, treating the solid surface with a secreted Dkk protein or epitope-tagged Dkk and a test compound, and determining whether the compound regulates binding between Dkk and LRP5/LRP6/HBM using antibodies to Dkk or the epitope tag, or by directly measuring the activity of an epitope tag. The epitope tag is alkaline phosphatase, histidine, or a V5 tag. Identifying binding partners for a Dkk protein comprises exposing the Dkk protein or LRP5/LRP6 binding fragment to a potential binding partner, and determining if the potential binding partner binds to a Dkk protein or the LRP5/LRP6 binding fragment. Detecting a modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide-binding pair that binds through extracellular interaction in their natural environment, comprises: (a) culturing at least one eukaryotic cell comprising a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or its segment joined to a transcriptional activation protein DNA binding domain, a nucleotide sequence encoding a second heterologous fusion protein comprising a second peptide or its segment joined to a transcriptional activation protein transcriptional activation domain, where binding of the first and second peptides reconstitutes a transcriptional activation protein, and a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, where expression of the reporter element produces a selected phenotype; (b) incubating the eukaryotic cell in the presence of a compound to detect the selected phenotype; and (c) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining if the compound affects the expression of the reporter element which produces the selected phenotype. The first peptide is a Dkk peptide, and the second peptide is LRP5, HBM, LRP6 or Dkk-binding portion of LRP5/LRP6/HBM. Alternatively, the first peptide is a Dkk interacting protein or Dkk-binding fragment, and the second peptide is a Dkk peptide. The eukaryotic cell is a yeast cell such as *Saccharomyces*, preferably *Saccharomyces cerevisiae*. The Dkk is Dkk-1, and the compound comprises one or more Dkk interacting proteins or a Dkk-binding fragment. The compound is directly added to the assay or is recombinantly expressed by the eukaryotic cell in addition to the first and second peptides. The eukaryotic cell further comprises at least one endogenous nucleotide sequence encoding the DNA binding domain of a transcriptional activation protein, the transcriptional activation domain of a transcriptional activation protein or the reporter element, where at least one of the endogenous nucleotide sequences is inactivated by mutation or deletion. The peptide binding pair comprises a ligand and a receptor to which the ligand binds. The transcriptional activation protein is Gal4, Gnc4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16 or a mammalian nuclear receptor. Preferably at least one of the heterologous fusion proteins is expressed from an autonomously replicating plasmid. The DNA binding

domain is a heterologous DNA-binding domain of a transcriptional activation protein, and the DNA binding protein is a mammalian steroid receptor or bacterial LexA protein. The reporter element is a LacZ, a polynucleotide encoding luciferase, a polynucleotide encoding green fluorescent protein, or a polynucleotide encoding chloramphenicol acetyltransferase, preferably LacZ. The test sample comprises an LRP5 peptide aptamer, preferably OST262, or an LRP5 antibody. Identifying potential compounds which modulate Dkk activity comprises measuring the effect on binding of one or more Dkk interacting proteins or a Dkk-binding fragment, with a Dkk or its fragment in the presence or absence of a compound, and identifying as a potential Dkk modulatory compound a compound which modulates the binding between one or more Dkk interacting proteins or Dkk-binding fragment, and Dkk or its fragment. Identifying Dkk interacting proteins, which modulate the interaction of Dkk with the Wnt signaling pathway, comprises injecting Dkk and potential Dkk interacting protein mRNA into a *Xenopus* blastomere, assessing axis duplication or marker gene expression, and identifying compositions which elicit changes in axis duplication or marker gene expression as Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway. The mRNA of HBM, LRP5/6, any wnt, Wnt antagonist, Wnt pathway modulator, or a combination of these is co-injected into the *Xenopus* blastomere. The marker gene analyzed is Siamois, Xnr3, slug, Xbra, HNK-1, endodermin, Xlhbox8, BMP2, %%%BMP4%%% , XLRP6, EF-1 or ODC. The method alternatively comprises transfected cells with constructs containing Dkk and potential Dkk interacting proteins, assessing changes in expression of a reporter gene linked to a Wnt-responsive promoter, and identifying as a Dkk interacting protein in any protein which alters reporter gene expression compared with cells transfected with a Dkk construct alone. The cells are HOB-03-CE6, HKE293 or U2OS cells. The Wnt-responsive promoter is TCF or LEF. The cells are co-transfected with cytomegalovirus (CMV) beta-galactosidase. Identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway comprises transfected cells with constructs containing Dkk and Wnt proteins, assessing changes in expression of a reporter element linked to a Wnt-responsive promoter, and identifying as Dkk/Wnt interaction modulating compound any compound which alters reporter gene expression compared to cells transfected with a Dkk construct alone. Wnt3a and Wnt1 constructs are co-transfected into the cells, where the cells are HOB-03-CE6, HKE293 or U2OS cells. The reporter element is TCF-luciferase and/or tk-Renilla. Testing compounds that modulate Dkk-mediated activity in a mammal comprises providing a group of transgenic animals having a regulatable one or more Dkk genes, a knock-out of Dkk genes or a knock in of one or more Dkk genes, providing a second group of control animals respectively for the group of transgenic animals, exposing the animals to a potential Dkk-modulating compound which modulates bone mass or lipid levels, and comparing the transgenic animals and the control group of animals and determining the effect of the compound on bone mass or lipid levels in the transgenic animals compared to the control animals. Screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprises exposing a Dkk interacting proteins or a Dkk-binding fragment to a compound, and determining whether the compound binds to a Dkk interacting proteins or Dkk-binding fragment. Modulation is determined if the compound binds to the Dkk interacting protein or the Dkk binding fragment. Preferred Composition: The composition of (7) comprises an LRP5, LRP6 or HBM activity-modulating compound that binds to Dkk thus modulating the interaction of Dkk with LRP5, LRP6 or HBM. The LRP5-, LRP6- or HBM-modulating compound comprises one or more Dkk interacting proteins and Dkk-binding fragments, a monoclonal antibody or its immunologically active fragment that binds to a Dkk interacting protein or Dkk binding fragment, an antisense, a siRNA or shRNA molecule that recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins, a Dkk peptide aptamer, a Dkk interacting protein peptide aptamer or its mimetic, an LRP5 peptide aptamer, preferably OST262, or an LRP5 antibody. ACTIVITY - Osteopathic; Antiinflammatory; Antiarthritic. No biological data is given. MECHANISM OF ACTION - Dkk modulator. USE - The method is useful for modulating lipid levels and/or bone mass, and is useful in treating or diagnosing abnormal lipid levels and bone mass disorders, such as osteoporosis, bond fracture, age-related loss of

bone, a chondrodyrophy, drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, Paget's disease, osteoarthritis, and rickets. Modulators of Dkk activity are useful for as reagents in studying bone mass and lipid level modulation, in modulating Wnt signaling, or treating Dkk-mediated disorders. ADMINISTRATION - Dosage is 0.0001-50, preferably 0.1-1 mg/kg. Administration can be parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal. EXAMPLE - No relevant examples are given. (173 pages)

? b 411;set files biotech

30jan09 17:33:07 User219511 Session D756.4

\$19.07 0.697 DialUnits File357

\$9.30 2 Type(s) in Format 7

\$9.30 2 Types

\$28.37 Estimated cost File357

\$0.53 TELNET

\$28.90 Estimated cost this search

\$32.52 Estimated total session cost 1.811 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)

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\*\*\* DIALINDEX search results display in an abbreviated \*\*\*

\*\*\* format unless you enter the SET DETAIL ON command. \*\*\*

You have 27 files in your file list.

(To see banners, use SHOW FILES command)

? s cg and (peptide or peptides or protein or proteins or polypeptide or polypeptides) and immobili?

Your SELECT statement is:

s cg and (peptide or peptides or protein or proteins or polypeptide or polypeptides) and immobili?

Items File

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2 34: SciSearch(R) Cited Ref Sci\_1990-2009/Jan W4

8 357: Derwent Biotech Res.\_1982-2008/Nov W5

1 370: Science\_1996-1999/Jul W3

3 files have one or more items; file list includes 27 files.

? save temp; b 34,357,370;exs;rd

Temp SearchSave "Tl646452431" stored

30jan09 17:34:24 User219511 Session D756.5

\$5.19 1.764 DialUnits File411

\$0.53 TELNET

\$5.72 Estimated cost this search

\$38.24 Estimated total session cost 3.575 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 34:SciSearch(R) Cited Ref Sci\_1990-2009/Jan W4

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File 357:Derwent Biotech Res.\_1982-2008/Nov W5

(c) 2008 Thomson Reuters

File 370:Science\_1996-1999/Jul W3

(c) 1999 AAAS

\*File 370: This file is closed (no updates). Use File 47 for more current information.

Set Items Description

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Executing Tl646452431

1851 CGG

361009 PEPTIDE

195289 PEPTIDES

1832177 PROTEIN

780168 PROTEINS

100271 POLYPEPTIDE

40510 POLYPEPTIDES

123310 IMMOBILI?

S1 11 CGG AND (PEPTIDE OR PEPTIDES OR PROTEIN OR PROTEINS OR POLYPEPTIDE OR POLYPEPTIDES) AND IMMOBILI?

S2 11 RD (unique items)

? t s2/7/1-11

2/7/1 (Item 1 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

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06145053 Genuine Article#: XX820 Number of References: 18

Title: Characterization of DNA probes %%%immobilized%%% on gold surfaces

Author(s): Herne TM (REPRINT) ; Tarlov MJ

Corporate Source: NATL INST STAND & TECHNOL,CHEM SCI & TECHNOL LAB/GAITHERSBURG//MD/20899 (REPRINT)

Journal: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 1997, V119, N38 (SEP 24), P8916-8920

ISSN: 0002-7863 Publication date: 19970924

Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036

Language: English Document Type: ARTICLE

Abstract: We have characterized thiol-derivatized, single-stranded DNA

(5'-HS-(CH<sub>2</sub>)<sub>6</sub>-CAC GAC GTT GTA AAA CGA %%%CGG%%% CCA G-3', abbreviated HS-ssDNA) attached to gold via a sulfur-gold linkage using X-ray photoelectron spectroscopy (XPS), ellipsometry, and P-32-radiolabeling experiments. We found that hybridization of surface-bound HS-ssDNA is dependent on surface coverage. The buffer concentration of the HS-ssDNA solution was found to have a profound effect on surface coverage, with adsorption greatly reduced at low salt concentration. More precise control over surface coverage was achieved by creating mixed monolayers of the thiol-derivatized probe and a spacer thiol, mercaptohexanol (MCH), by way of a two-step method, where first the gold substrate is exposed to a micromolar solution of HS-ssDNA, followed by exposure to a millimolar solution of MCH. A primary advantage of using this two-step process to form HS-ssDNA/MCH mixed monolayers is that nonspecifically adsorbed DNA is largely removed from the surface. Thus, the majority of surface-bound probes are accessible for specific hybridization with complementary oligonucleotides and are able to discriminate between complementary and noncomplementary target molecules. Moreover, the probe-modified surfaces were found to be stable, and hybridization reactions were found to be completely reversible and specific in a series of experiments where duplex melting was examined.

2/7/2 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

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02345614 Genuine Article#: KV760 Number of References: 21

Title: SCREENING FOR POINT MUTATIONS BY SEMIAUTOMATED DNA SEQUENCING USING SEQUENASE AND MAGNETIC BEADS

Author(s): LEREN TP; RODNINGEN OK; ROSBY O; SOLBERG K; BERG K

Corporate Source: ULLEVAL UNIV HOSP,DEPT MED GENET,POB 1036/N-0315

OSLO//NORWAY/; UNIV OSLO,INST MED GENET/OSLO 3//NORWAY/

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Language: ENGLISH Document Type: ARTICLE

Abstract: We have established an improved method for detecting point

mutations by semi-automated DNA sequencing of PCR fragments generated from genomic DNA. The method employs magnetic beads to create %%%immobilized%%% single-stranded DNA templates, and the sequencing reaction is performed with Sequenase(R). This method is superior to sequencing with Taq DNA polymerase because the uniform peak height with Sequenase makes heterozygosity easily detectable as double peaks that are half the normal height. Detection of heterozygosity by this method is illustrated by sequencing a 180-bp fragment of the human apolipoprotein B gene. This fragment contains codon 3500, where a point mutation (3500 %%%CGG%%%-->CAG) is found in subjects with the autosomal dominant disease familial defective apolipoprotein B. The nonuniform peak height with Taq DNA polymerase makes it more difficult to detect heterozygosity. This is also illustrated by sequencing a 278-bp

fragment of the low-density lipoprotein receptor gene.

2/7/3 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0383261 DBR Accession No.: 2005-28967 PATENT

New bone graft or scaffolding materials %%%immobilized%%% with osteogenesis enhancing %%%peptides%%% on the surface, useful for tissue engineering applications - cell adhesion-inducing %%%protein%%% and tissue growth factor-derived %%%protein%%% %%%immobilization%%% for use in tissue engineering

AUTHOR: PARK Y J; CHUNG C; LEE S J; RHEE S H

PATENT ASSIGNEE: UNIV SEOUL NAT IND FOUND 2005

PATENT NUMBER: WO 200589826 PATENT DATE: 20050929 WPI ACCESSION NO.: 2005-684044 (200570)

PRIORITY APPLIC. NO.: KR 19010 APPLIC. DATE: 20040319

NATIONAL APPLIC. NO.: WO 2005KR801 APPLIC. DATE: 20050318

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A bone graft material or a scaffold

for tissue engineering applications, which has a cell adhesion-inducing %%%peptide%%% and/or tissue growth factor-derived %%%peptide%%% %%%immobilized%%% on the surface, is new. BIOTECHNOLOGY - Preferred

Bone Graft Material/Scaffold: The cell adhesion-inducing %%%peptide%%% has an amino acid sequence of RGD. The cell adhesion-inducing %%%peptide%%% has an amino acid sequence of Cys-Gly-Gly-Arg-Gly-Asp-Ser

(SEQ ID NO: 1) or Cys-Gly-Gly-Val-Ala-Cys-Asp-Cys-Arg-Gly-Asp-Phe-Cys

(SEQ ID NO: 2). The tissue growth factor-derived %%%peptide%%% is at least one %%%peptide%%% selected from: (a) the amino acid sequence at

positions 2-18 of each of bone morphogenetic %%%proteins%%% (BMP)-2, 4 and 6 (SEQ ID NO: 3 for BMP-2, SEQ ID NO: 4 for BMP-4, and SEQ ID NO: 5 for BMP-6); the amino acid sequence at positions 16-34 of BMP-2 (SEQ ID NO: 6), the amino acid sequence at positions 47-71 (SEQ ID NO: 7), the amino acid sequence at positions 73-92 (SEQ ID NO: 8), the amino acid sequence at positions 88-105 (SEQ ID NO: 9), the amino acid sequence at positions 283-302 (SEQ ID NO: 10), the amino acid sequence at positions 335-353 (SEQ ID NO: 11) and the amino acid sequence at positions 370-390 (SEQ ID NO: 12); the amino acid sequence at positions 74-93 of BMP-4 (SEQ ID NO: 13), the amino acid sequence at positions 293-313 (SEQ ID NO: 14), the amino acid sequence at positions 360-379 (SEQ ID NO: 15) and the amino acid sequence at positions 382-402 (SEQ ID NO: 16); the amino acid sequence at positions 91-110 of BMP-6 (SEQ ID NO: 17), the amino acid sequence at positions 397-418 (SEQ ID NO: 18), the amino acid sequence at positions 472-490 (SEQ ID NO: 19) and the amino acid sequence at positions 487-510 (SEQ ID NO: 20); and the amino acid sequence at positions 98-117 of BMP-7 (SEQ ID NO: 21), the amino acid sequence at positions 320-340 (SEQ ID NO: 22), the amino acid sequence at positions 390-409 (SEQ ID NO: 23) and the amino acid sequence at positions 405-423 (SEQ ID NO: 24); (b) the amino acid sequence at positions 62-69 of bone %%%sialoprotein%%% (SEQ ID NO: 25), the amino acid sequence at positions 139-148 (SEQ ID NO: 26), the amino acid sequence at positions 259-277 (SEQ ID NO: 27), the amino acid sequence at positions 199-204 (SEQ ID NO: 28), the amino acid sequence at positions 151-158 (SEQ ID NO: 29), the amino acid sequence at positions 275-291 (SEQ ID NO: 30), the amino acid sequence at positions 20-28 (SEQ ID NO: 31), the amino acid sequence at positions 65-90 (SEQ ID NO: 32), the amino acid sequence at positions 150-170 (SEQ ID NO: 33) and the amino acid sequence at positions 280-290 (SEQ ID NO: 34); (c) the amino acid sequence at positions 242-250 of a transforming growth factor (SEQ ID NO: 35), the amino acid sequence at positions 279-299 (SEQ ID NO: 36) and the amino acid sequence at positions 343-361 (SEQ ID NO: 37); (d) the amino acid sequence at positions 100-120 of a platelet-derived (SEQ ID NO: 37) and the amino acid sequence at positions 121-140 (SEQ ID NO: 39); (e) the amino acid sequence at positions 23-31 of an acidic fibroblast (SEQ ID NO: 40) and the amino acid sequence at positions 97-105 (SEQ ID NO: 41); (f) the amino acid sequence at positions 16-27 of a basic fibroblast growth factor (SEQ ID NO: 42), the amino acid sequence at positions 37-42 (SEQ ID NO: 43), the amino acid sequence at positions 78-84 (SEQ ID NO: 44) and the

amino acid sequence at positions 107-112 (SEQ ID NO: 45); (g) the amino acid sequence at positions 255-275 of dentin %%%sialoprotein%%% (SEQ ID NO: 46), the amino acid sequence at positions 475-494 (SEQ ID NO: 47) and the amino acid sequence at positions 551-573 (SEQ ID NO: 48); (h) the amino acid sequence at positions 63-83 of a heparin binding EGF-like growth factor (SEQ ID NO: 49), the amino acid sequence at positions 84-103 (SEQ ID NO: 50), the amino acid sequence at positions 104-116 (SEQ ID NO: 51) and the amino acid sequence at positions 121-140 (SEQ ID NO: 52); (i) the amino acid sequence at positions 326-350 of the cadherin EGF LAG seven-pass G-type receptor 3 (SEQ ID NO: 53), the amino acid sequence at positions 351-371 (SEQ ID NO: 54), the amino acid sequence at positions 372-400 (SEQ ID NO: 55), the amino acid sequence at positions 401-423 (SEQ ID NO: 56), the amino acid sequence at positions 434-545 (SEQ ID NO: 57), the amino acid sequence at positions 546-651 (SEQ ID NO: 58), the amino acid sequence at positions 1375-1433 (SEQ ID NO: 59), the amino acid sequence at positions 1435-1471 (SEQ ID NO: 60), the amino acid sequence at positions 1475-1514 (SEQ ID NO: 61), the amino acid sequence at positions 1515-1719 (SEQ ID NO: 62), the amino acid sequence at positions 1764-1944 (SEQ ID NO: 63) and the amino acid sequence at positions 2096-2529 (SEQ ID NO: 64); or (j) the amino acid sequence at positions 54-159 of an osteoblast specific amino acid sequence at cadherin (OB-cadherin) (SEQ ID NO: 65), the positions 160-268 (SEQ ID NO: 66), the amino acid sequence at positions 269-383 (SEQ ID NO: 67), the amino acid sequence at positions 384-486 (SEQ ID NO: 68) and the amino acid sequence at positions 487-612 (SEQ ID NO: 69), where SEQ ID NOS: 3-69 comprises 6-205 amino acids. The tissue growth factor-derived %%%peptide%%% has an addition of cysteine at N-terminal end, where the addition of cysteine is %%%CGG%%% spacer type. The bone graft material is any one selected from organism-derived bone mineral powders and porous blocks originated from autogenous bone, bovine bone and porcine bone, synthetic hydroxyapatite powders and porous blocks, tricalcium phosphate powders and porous blocks, monocalcium phosphate powders and porous blocks, bone graft materials made of silicon dioxide (silica), bonepacking graft materials made of a mixture of silica and polymer, fine particles and porous scaffolds made of biocompatible polymers, including chitosan and polylactic acid, and titanium and three-dimensional scaffolds. The surface of the bone graft material is %%%immobilized%%% with a crosslinker selected from 1,4-bis-maleimidobutane (BMB), 1,11-bis-maleimido tetraethyleneglycol (BM(PEO)4), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), succinimidyl-4- (N-maleimido methylcyclohexane-1-c arboxy-(6amidocaproate)) (SMCC) and sulfo-SMCC, succinimidyl 6-(3-(2-pyridylthio)-ropionamido) hexanoate (SPDP) and sulfo-SPDP, m-maleimidobenzoyl-Nhydroxysuccinimide ester (MBS) and sulfo-MBS, succinimidyl (4-(p-maleimidophenyl) butyrate) (SMPB) or sulfo-SMPB. The scaffold for tissue engineering applications is a barrier membrane or an implant, where the barrier membrane is porous membranes made of polylactic acid; regeneration membranes made of nanofibers of chitin or chitosan; or film-shaped barrier membranes made of chitin or chitosan, and where the implant is a titanium implant. The surface of the implants is modified by oxidation and nitrification so as to facilitate the adhesion of the active %%%peptide%%% to the surface. USE - The bone graft material or a scaffold is useful for tissue engineering applications.(75 pages)

2/7/4 (Item 2 from file: 357)

DIALOG(R)File 357:Dowert Biotech Res.  
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0352075 DBR Accession No.: 2004-24367 PATENT

New isolated recombinant nucleic acid encoding %%%polypeptides%%% having pectate lyase activity, useful in treating plant cell walls, textile scouring, manufacturing feed or food and aiding digestion - recombinant enzyme %%%protein%%% production via plasmid expression in host cell for use in plant engineering

AUTHOR: KEROVUO J; SOLBAK A; GRAY K; MCCANN R; PUROHIT S; GERENDASH J; JANSEN G; DAHOD S

PATENT ASSIGNEE: DIVERSA CORP 2004

PATENT NUMBER: WO 200490099 PATENT DATE: 20041021 WPI ACCESSION NO.:

2004-748745 (200473)

PRIORITY APPLIC. NO.: US 484798 APPLIC. DATE: 20030703

NATIONAL APPLIC. NO.: WO 2004US10229 APPLIC. DATE: 20040402

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated recombinant nucleic acid

(I) comprises a sequence at least 50% identical to any of 67 fully defined sequences of 972-3618 bp, designated as N1-N67, over a region of at least about 100 residues, where the nucleic acid encodes at least one %%%polypeptide%%% having a pectate lyase activity. DETAILED DESCRIPTION - An isolated recombinant nucleic acid (I) comprises a sequence at least 50% identical to any of 67 fully defined sequences of 972-3618 bp (all odd SEQ ID NOS. from SEQ ID NO: 1-133), designated as N1-N67, over a region of at least about 100 residues, where the nucleic acid encodes at least one %%%polypeptide%%% having a pectate lyase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection. INDEPENDENT CLAIMS are also included for the following: (1) an isolated (1a) or recombinant nucleic acid, where the nucleic acid comprises a sequence that hybridizes under stringent conditions to any of nucleotide sequences N1-N67, where the nucleic acid encodes a %%%polypeptide%%% having a pectate lyase activity; (2) a nucleic acid probe for identifying a nucleic acid encoding a %%%polypeptide%%% with a pectate lyase activity, where the probe comprises at least 10 consecutive bases from any of nucleotide sequences N1-N67, where the probe identifies the nucleic acid by binding or hybridization; (3) an amplification primer sequence pair for amplifying a nucleic acid encoding a %%%polypeptide%%% having a pectate lyase activity, where the primer pair is capable of amplifying (I) or (Ia); (4) amplifying a nucleic acid encoding a %%%polypeptide%%% having a pectate lyase activity, comprising an amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying (I) or (Ia); (5) an expression cassette comprising a nucleic acid comprising (I) or (Ia); (6) a vector comprising (I) or (Ia); (7) a transformed cell comprising (I) or (Ia); (8) a transgenic non-human animal comprising (I) or (Ia); (9) a transgenic plant comprising (I) or (Ia); (10) a transgenic seed comprising (I) or (Ia); (11) an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to (I) or (Ia); (12) a double-stranded inhibitory RNA (iRNA) molecule comprising (I) or (Ia); (13) inhibiting the translation of a pectate lyase message in a cell, comprising administering to the cell or expressing in the cell an antisense oligonucleotide or a double-stranded iRNA; (14) an isolated or recombinant %%%polypeptide%%% (II) having at least 50% sequence identity to any of 67 fully defined sequences of 323-1205 amino acids (all even SEQ ID NOS from SEQ ID NO: 2-134, designated as P1-P67, over a region of at least about 100 residues, or encoded by (I) or (Ia)); (15) a %%%protein%%% preparation comprising (II), comprising a liquid, solid or gel; (16) a heterodimer comprising (II) and a second domain; (17) a homodimer comprising (II); (18) an %%%immobilized%%% %%%polypeptide%%% comprising (II) or its subsequence; (19) an array comprising the %%%immobilized%%% %%%polypeptide%%%, (I) or (Ia); (20) an isolated or recombinant antibody that specifically binds to (II); (21) a hybridoma comprising the antibody; (22) isolating (M1) or identifying a %%%polypeptide%%% with a pectate lyase activity; (23) making (M2) an anti-pectate lyase antibody; (24) producing (M3) a recombinant %%%polypeptide%%%; (25) identifying (M4) a %%%polypeptide%%% having a pectate lyase activity; (26) identifying (M5) a pectate lyase substrate; (27) determining (M6) whether a test compound specifically binds to a %%%polypeptide%%% (II); (28) identifying (M7) a modulator of a pectate lyase activity; (29) a computer system; (30) identifying (M8) a feature in a sequence comprising (II) or encoded by (I) or (Ia); (31) comparing (M9) a first sequence to a second sequence; (32) isolating (M10) or recovering a nucleic acid encoding a %%%polypeptide%%% with a pectate lyase activity from an environmental sample; (33) generating (M11) a variant of a nucleic acid encoding a %%%polypeptide%%% with a pectate lyase activity; (34) modifying (M12) codons in a nucleic acid encoding a %%%polypeptide%%% with a pectate lyase activity to modulate its expression in a host cell; (35) producing (M13) a library of nucleic

acids encoding a plurality of modified pectate lyase active sites or substrate binding sites; (36) making or modifying (M14) a small molecule; (37) determining (M15) a functional fragment of a pectate lyase enzyme; (38) whole cell engineering (M16) of new or modified phenotypes by using real-time metabolic flux analysis; (39) an isolated or recombinant signal sequence comprising specific residues of amino acid sequences P1-P67; (40) a chimeric %%%polypeptide%%% ; (41) an isolated or recombinant nucleic acid encoding the chimeric %%%polypeptide%%% ; (42) increasing M17) thermotolerance or thermostability of a pectate lyase %%%polypeptide%%% ; (43) overexpressing (M18) a recombinant pectate lyase in a cell; (44) making (M19) a transgenic plant; (45) expressing (M20) a heterologous nucleic acid sequence in a plant cell; (46) hydrolyzing (M21), breaking up or disrupting a phospholipid/pectin comprising composition; (47) a detergent composition comprising (II) or a %%%polypeptide%%% encoded by (I) or (la); (48) a textile or fabric comprising (II); (49) fiber, thread, textile or fabric scouring, comprising providing a %%%polypeptide%%% having a pectate lyase activity; (50) a feed or a food comprising (II); (51) a paper or paper product or paper pulp comprising (II); (52) a pharmaceutical composition or oral care product comprising (II); (53) ameliorating (M22) soft-rot spoilage by slowing the normal growth of the powdery mildew pathogen Erysiphe cichoracearum in a plant or plant part; (54) an isolated or recombinant nucleic acid comprising a sequence modification of N1-N67; (55) an isolated or recombinant %%%polypeptide%%% comprising a sequence modification of P1-P67; (56) generating (M24) a modified pectate-lyase encoding nucleic acid (see above); (57) generating a modified pectate lyase; (58) formulation for treating a material with a pectate lyase comprising a dosage of pectate lyase in the range of 1-100 grams per ton per ton treated material; and (59) a bioscouring process (M23). BIOTECHNOLOGY - Preferred Nucleic Acid: The sequence identity of (I) is at least about 51-99%, or more, or is 100% sequence identity to nucleotide sequences N1-N67. The sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript. The nucleic acid encodes a %%%polypeptide%%% having any of amino acids P1-P53. The sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d nr pataa -F F, and all other options are set to default. The pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate). The pectate lyase activity comprises the breakup or dissolution of plant cell walls, and beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid, and/or catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid. The pectate lyase activity is exo-acting or endo-acting, or is endo-acting and acts at random sites within a polymer chain to give a mixture of oligomers, or is exo-acting and acts from one end of a polymer chain and produces monomers or dimers. The pectate lyase activity further catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination or hydrolysis, and comprises activity the same or similar to pectate lyase (EC 4.2.2.2), poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67),exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC 3.2.1.82). The pectate lyase activity also comprises beta-elimination (trans-elimination) or hydrolysis of galactan to galactose or galactooligomers, and/or beta-elimination (trans-elimination) or hydrolysis of a plant fiber that is cotton fiber, hemp fiber or flax fiber. The pectate lyase activity is thermostable or thermotolerant. The %%%polypeptide%%% retains a pectate lyase activity under conditions comprising a temperature range of 37degreesC to 95degreesC. The isolated or recombinant nucleic acid of (I) is at least 50-1000 or more residues in length or the full length of the gene or transcript. The stringent conditions include a wash step comprising a wash in 0.2 x SSC at a temperature of about 65degreesC for about 15 minutes. The amplification of the pectate lyase-encoding nucleic acid is by polymerase chain

reaction (PCR). The nucleic acid is generated by amplification of a gene library that is an environmental library. Also an isolated or recombinant nucleic acid having a sequence comprising a sequence modification of N1-N67, where the sequence modification comprises one or more changes in the nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO: 131 are changed to CAT or CAC; 544 to 546 to GTG, GTT, GTC, or GTA; 568 to 570 to TTG, TTA, CTT, CTC, CTA, or CTG; 589 to 591 to GGT, GGC, GGA, or GGG; 622 to 624 to AAG or AAA; 655 to 657 to ATG; 667 to 669 to GAG or GAA; 763 to 765 to %%%CGG%%% ; CGT, CGC, CGA, AGA, AGG; 787 to 789 to AAG or AAA; 823 to 825 to TAT or TAG; 925 to 927 to TGG; 934 to 936 to GTT, GTG, GTC, or GTA. Preferred Probe: The nucleic acid probe further comprises an oligonucleotide comprising at least 10-150 consecutive bases. Preferred Primer Pair: The amplification primer sequence pair comprises an oligonucleotide comprising at least 10 to 50 or 12-25 consecutive bases of the sequence. Also a first member having a sequence of (the 5') 15-25 residues from any of nucleotide sequences N1-N67, and a second member having a sequence of (the 5') 15-30 or more residues of the complementary strand of the first member. Preferred Cloning Vehicle: The cloning vehicle comprising (I) comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector in the cloning vehicle comprises an adenovirus, a retroviral or an adeno-associated viral vector. The vehicle further comprises a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage Pi-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC). Preferred Signal Sequence: The isolated or recombinant signal sequence comprises a sequence as set forth in residues 1-44 of any of amino acid sequences P1-P67. Preferred Cell: The transformed cell is a bacterial, mammalian, fungal, yeast, insect or a plant cell. Preferred Transgenic Animal: The animal is preferably a mouse. Preferred Plant: The transgenic plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, a cottonseed, a palm, a sesame plant, a peanut plant, a sunflower plant or a tobacco plant. Preferred Seed: The transgenic seed also comprises the above. Preferred RNAi Molecule: The double-stranded inhibitory RNA (RNAi) molecule is about 15-25 or more duplex nucleotides in length. Preferred %%%Polypeptide%%% : The sequence identity of (II) is at least about 51-99%, or more, or has 100% sequence identity to nucleotide sequences N1-N67. The sequence identity is over a region of at least about 50-1050, or more residues, or the full length of an enzyme. The pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate). The pectate lyase activity comprises the breakup or dissolution of plant cell walls, and beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid, and/or catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid. The %%%polypeptide%%% retains a pectate lyase activity under conditions comprising a temperature range of 37degreesC to 95degreesC. The thermotolerance in the isolated or recombinant %%%polypeptide%%% comprises retention of at least half of the specific activity of the pectate lyase at 37degreesC after being heated to an elevated temperature, or comprises retention of specific activity at 37degreesC in the range of 500-1200 units per milligram of %%%protein%%% after being heated to an elevated temperature. The %%%polypeptide%%% comprises at least one glycosylation site, where the glycosylation is an N-linked glycosylation. The %%%polypeptide%%% is glycosylated after being expressed in an *P. pastoris* or an *S. pombe*. The %%%polypeptide%%% retains a pectate lyase activity under conditions comprising about pH 6.5-4.0, or under conditions comprising about pH 7.5-10.5. The %%%immobilized%%% %%%polypeptide%%% is %%%immobilized%%% on a cell, metal, resin, polymer, ceramic, glass, microelectrode, graphitic particle, bead, gel, plate, array or a capillary tube. The heterologous %%%polypeptide%%% or %%%peptide%%% in the chimeric %%%polypeptide%%% is not a pectate lyase, and is amino terminal to, carboxy terminal to or on both ends of the signal %%%peptide%%% (SP) or a catalytic domain (CD). The recombinant %%%polypeptide%%% comprising (II), may also lack a signal sequence, or have a heterologous signal sequence. Also an

isolated or recombinant %%%polypeptide%%% having a sequence comprising a sequence modification of P1-P67, where the sequence modification comprises one or more changes in the amino acid of SEQ ID NO: 132 at the equivalent of the alanine at residue 118 to a histidine; alanine at residue 182 to a valine; threonine at residue 190 to leucine; alanine at residue 197 to glycine; serine at residue 208 to lysine; threonine at residue 219 to methionine; threonine at residue 223 to glutamic acid; serine at residue 255 to arginine; serine at residue 263 to lysine; asparagine at residue 275 to a tyrosine; tyrosine at residue 309 to tryptophan; or serine at residue 312 changed to valine. Preferred Chimeric %%%Polypeptide%%%: This comprises at least a first domain comprising signal %%%peptide%%% (SP) from any of amino acid sequences P1-P67, and a second domain comprising a heterologous %%%polypeptide%%% or %%%peptide%%%, where the heterologous %%%polypeptide%%% or %%%peptide%%% is not naturally associated with the signal %%%peptide%%% (SP). Preferred Heterodimer: The second domain of the heterodimer is a %%%polypeptide%%% and the heterodimer is a fusion %%%protein%%%, or is an epitope or a tag. Preferred Methods: (M1) comprises providing a sample comprising %%%polypeptides%%%, and contacting the sample with an antibody under conditions where the antibody can specifically bind to the %%%polypeptide%%%; (M2) comprises administering to a non-human animal (I), (Ia), or (II), or their subsequence, to generate a humoral immune response. (M3) further comprises transforming a host cell with the nucleic acid followed by expressing the nucleic acid. (M3) comprises providing (I) or (Ia) operably linked to a promoter, and expressing under conditions that allow expression of the %%%polypeptide%%%. (M4) comprises providing (II), a pectate lyase substrate, and contacting the %%%polypeptide%%% with the substrate and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product. (M5) comprises providing (II), a test substrate, and contacting the %%%polypeptide%%% with the test substrate and detecting a decrease in the amount of substrate or an increase in the amount of reaction product. (M6) comprises providing (II) or expressing (I) or (Ia) or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a %%%polypeptide%%%, contacting the %%%polypeptide%%% with the test compound, and determining whether the test compound specifically binds to the %%%polypeptide%%% . (M7) comprises providing a pectate lyase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product, in reactions with or without a test compound and comparing substrate or reaction product amounts. In (M10) each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least 10-50 consecutive bases of nucleotide sequences N1-N67. Also treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair, combining the nucleic acid with the amplification primer pair and amplifying the nucleic acid from the environmental sample. The sample comprises a water, liquid, soil, air or a biological sample. The biological sample is derived from a bacterial, protozoan, insect, yeast, plant, fungal or mammalian cell. Generating a variant of nucleic acid (M11) or producing a library of nucleic acids further comprises expressing the variant nucleic acid to generate a variant pectate lyase %%%polypeptide%%%. The modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis et cetera. The method is iteratively repeated until a pectate lyase having an altered or different activity/stability from that of a %%%polypeptide%%% encoded by the template nucleic acid is produced. The variant pectate lyase %%%polypeptide%%% is thermostolerant, and retains some activity after being exposed to an elevated temperature, or has increased glycosylation as compared to the pectate lyase encoded by a template nucleic acid, or has a pectate lyase activity under a high temperature, where the pectate lyase encoded by the template nucleic acid is not active under the high temperature. The method is also iteratively repeated until a pectate lyase coding sequence having an altered codon usage from that of the template nucleic acid is produced, or until a

pectate lyase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced. The method further comprises compassing a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions, and testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library. Testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity. (M12) comprises providing a nucleic acid encoding a %%%polypeptide%%% with a pectate lyase activity comprising any of nucleotide sequences N1-N67, and identifying a non-preferred or a less preferred codon in the nucleic acid and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, and vice versa for decreasing expression in a host cell. In (M13) the modified active sites or substrate binding sites are derived from a first nucleic acid sequence comprising a sequence that hybridizes under stringent conditions to any of N1-N67 encoding a first pectate lyase active site or substrate binding site, and using a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acids to generate a set of active site-encoding or substrate-binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized. (M14) comprises providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, where one of the enzymes comprises a pectate lyase enzyme encoded by (I) or (Ia), providing a substrate, and reacting the substrate with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule. (M15) comprises providing a pectate lyase enzyme, deleting amino acid residues from the sequence and testing the remaining subsequence for a pectate lyase activity. (M16) comprises modifying by addition to the cell of a nucleic acid comprising (I) or (Ia) , culturing the modified cell to generate a plurality of modified cells, measuring at least one metabolic parameter of the cell by monitoring the cell culture in real time, and analyzing the data to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions. (M17) comprises glycosylating a pectate lyase, where the %%%polypeptide%%% comprises at least thirty contiguous amino acids of (II), or a %%%polypeptide%%% encoded by (I) or (Ia). (M18) comprises expressing a vector comprising (I) or (Ia) where overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector. In (M18) the genetic composition of the cell is modified deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene. The method further comprises selecting a cell comprising a newly engineered phenotype, and culturing the selected cell. (M19) comprises introducing a heterologous nucleic acid sequence from N1-N67 into the cell, producing a transformed plant cell, and producing a transgenic plant from the transformed cell. (M19) comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts, and introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host. The pectate lyase in fiber, thread, textile or fabric scouring is an alkaline active and thermostable pectate lyase. The method further comprises addition of an alkaline and thermostable amylase .The desizing and scouring treatments are combined in a single bath. The method also comprises addition of an alkaline and thermostable amylase in the contacting step. (M20) comprises transforming the plant cell with a heterologous nucleic acid sequence selected from N1-N67, operably linked to a promoter, growing the plant under conditions where the heterologous nucleic acid sequence is expressed in the plant cell. (M21) comprises contacting (I) and a composition comprising a

phospholipid under conditions where the pectate lyase hydrolyzes, breaks up or disrupts the composition, also liquefying or removing a pectin or pectate (polygalacturonic acid) from a composition. The composition comprises a plant or bacterial cell wall. The plant is a cotton, hemp or flax plant. (M22) comprises administering a composition that decreases the expression or activity of a pectate lyase in the plant or plant part, where the composition comprises an antibody, or an antisense oligonucleotide, a ribozyme or an RNAi comprising an antisense oligonucleotide complementary to or capable of hybridizing under stringent conditions to a sequence of (I). The composition in ameliorating soft-rot spoilage is sprayed onto the plant or plant part. (M23) comprises providing a pectate lyase as cited, providing a pectin- or polygalacturonic acid-comprising material, contacting the pectate lyase with the material under conditions comprising about pH 8.5, in bicarbonate buffer, comprising a non-ionic wetting agent at, about 1 g/L, where the pectate lyase ratio in an enzyme bath is between about 10:1 to 50:1 L pectate lyase:kg of material, where the pectate lyase dose is 0.1-0.2 ml of a concentrated extract per kg of material, or equivalent, at a temperature range of 500-700degreesC, and a treatment time of 20 minutes. (M23) comprises providing a pectate lyase as cited, providing a pectin- or polygalacturonic acid-comprising material, contacting the pectate lyase with the material under conditions pH 8.5, in bicarbonate buffer, comprising a non-ionic wetting agent at, about 1 g/L, where the pectate lyase ratio in an enzyme bath is between about 10:1 to 50:1 L pectate lyase:kg of material, where the pectate lyase dose is 0.1-0.2 ml of a concentrated extract per kg of material, or equivalent, at a temperature range of 500-700degreesC, and a treatment time of 20 minutes. The desizing or scouring treatments comprise conditions of pH 8.5 to pH 10.0 and temperatures of at 400degreesC. The method additionally comprises addition of a bleaching step, where the desizing, scouring and bleaching treatments are done simultaneously or sequentially in a single-bath container. The bleaching treatment comprises hydrogen peroxide and at least one bleach activator. The fiber, thread, textile or fabric comprises a cellulosic material that is a crude fiber, a yarn, a woven or knit textile, a cotton, a linen, a flax, a ramie, a rayon, a hemp, a jute or a blend of natural or synthetic fibers. (M24) comprises making one or more sequence modifications to a pectate-lyase encoding nucleic acid as detailed. Preferred Pharmaceutical Composition: The pharmaceutical composition acts as a digestive aid. Preferred Oral Product: The oral care product comprises a toothpaste, a dental cream, a gel or a tooth powder, an odontic, a mouth wash, a pre- or post brushing rinse formulation, a chewing gum, a lozenge or a candy. Preferred Detergent: The pectate lyase is a non-surface-active pectate lyase or a surface-active pectate lyase, and is formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form. Preferred Computer System: The computer system further comprises a sequence comparison algorithm and a data storage device having at least one reference sequence stored. The sequence comparison algorithm comprises a computer program that indicates polymorphisms. Preferred Formulation: The dosage of the formulation is 10-90 grams per ton, preferably 20-80 gram per ton, 30-70 grams per ton, or 40-50 grams per ton. The dosage can also be between 1-100 or more microg per gram, preferably 10-90 microg per gram, 20-80 microg per gram, 30-70 microg per gram, or 40-50 microg per gram. The dosage is also 0.5-50 or more mg per pound, between 1-45 mg per pound, 5-40 mg per pound, 10-35 mg per pound or 15-0 mg per pound. The formulation comprises a fabric or a cloth, and is a water-based formulation. The dosage further comprises an enzyme strength of 500-30000 units/ml. The formulation also comprises a lyophilized enzyme resuspended in water, and a glycerol, sucrose, sodium chloride, dextrin, propylene glycol, sorbitol, sodium sulfate or TRIS, or an equivalent, and a buffer comprising pH 7, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 35% glycerol, 300 ppm proxel; pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 10% sodium chloride, 25% glycerol, 300 ppm proxel; pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 5.5, 35% glycerol, 300 ppm proxel; pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; or, 20mM acetate buffer, pH 5.5, 35% glycerol; 20 mM MOPS, pH 7 or 25

mM MOPS, 50 mM NaCl, pH 7.5; pH 5.0, 40mM TRIS; pH 7.0, 40mM TRIS; pH 8.0, 40mM TRIS; pH 7.5, 50% glycerol; pH 7.5, 20% NaCl; pH 7.5, 30% propylene glycol; pH 7.5, 100mM sodium sulfate; pH 5.5, 35% glycerol. Preferred Biosourcing Process: The material in the biosourcing process comprises a fabric or a cloth. The pectate lyase dose is 0.137 ml of a concentrated extract per kg of material, or its equivalent. The contacting step further comprises use of a chelant, wherein the chelant is excluded from the enzyme bath and is added after 20 minutes of enzyme treatment and retained for 10 minutes before discharging bath. ACTIVITY - Gastrointestinal-Gen; Periodontal; Plant Growth Regulant; Antifungal. No biological data given. MECHANISM OF ACTION - Lyase-Modulator. USE - The methods and compositions of the present invention are useful in the field of molecular and cellular biology, biochemistry and biotechnology, in particular for using %%%polypeptides%%% having pectate lyase activity in treating plant cell walls, textile scouring, washing objects, extracting oils e.g., soybean oil, treating pa

2/7/5 (Item 3 from file: 357)

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0342507 DBR Accession No.: 2004-14799 PATENT

New nucleic acid comprising a codon-optimized nucleotide sequence encoding a component of a bacterial luciferase system, useful for developing a mammalian bioluminescence bioreporter for medical research and diagnostic applications - bacterium enzyme system and codon usage for bioluminescent reporter and medical diagnosis

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PATENT ASSIGNEE: UNIV TENNESSEE RES FOUND 2004

PATENT NUMBER: WO 200442010 PATENT DATE: 20040521 WPI ACCESSION NO.: 2004-400665 (200437)

PRIORITY APPLIC. NO.: US 422467 APPLIC. DATE: 20021030

NATIONAL APPLIC. NO.: WO 2003US34468 APPLIC. DATE: 20031030

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A nucleic acid comprising a codon-optimized nucleotide sequence encoding a component of a bacterial luciferase system, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a cell comprising the nucleic acid; and (2) introducing the codon-optimized nucleic acid into a mammalian cell. BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid comprises a codon-optimized nucleotide sequence that differs from a wild type nucleotide sequence that encodes the component of a bacterial luciferase system by at least one codon system selected from: TTT to TTC; TTA, CTA, TTG and CTT to CTG or CTC; ATT and AATA to ATC; GTT and GTA to GTG or GTC; TCT, TCA, and TCG to TCC; CCA and CCG to CCC or CCT; ACT, ACA and ACG to ACC; GCA and CGC to GCT or GCC; TAT to TAC; CAT to CAC; CAA to CAG; AAT to AAC; AAA to AAG; GAT to GAC; GAA to GAG; TGT to TGC; CGT and CGA to CGC, %%%CGG%%% and AGA; AGT to AGC; and GGT and GGA to GGC or GGG. The component of a bacterial luciferase system comprises a LuxA or LuxB %%%polypeptide%%% . The codon-optimized nucleotide sequence is 1084 or 984 bp, fully defined in the specification. The nucleic acid further comprises a regulatory element operably linked to the codon-optimized nucleotide sequence. The regulatory element comprises an enhancer. Preferred Cell: The cell is a mammalian cell. It is preferably %%%immobilized%%% on a substrate. USE - The nucleic acid is useful for developing a mammalian bioluminescence bioreporter for medical research and diagnostic applications. (43 pages)

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DIALOG(R)File 357.Derwent Biotech Res.

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0324079 DBR Accession No.: 2003-25219 PATENT

Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, useful for pre- or postnatal diagnosis of diseases, comprises extending the detection primer using labeled nucleotide triphosphates - DNA primer extension for use in disease

diagnosis and plant and animal breeding

AUTHOR: SODERLUND H E; SYVANEN A

PATENT ASSIGNEE: SODERLUND H E; SYVANEN A 2003

PATENT NUMBER: US 20030082530 PATENT DATE: 20030501 WPI ACCESSION NO.: 2003-708522 (200367)

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NATIONAL APPLIC. NO.: US 465322 APPLIC. DATE: 19950605

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Detecting (M1) a specific nucleotide

variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprising extending the detection primer using a polymerizing agent in a mixture containing one or more nucleoside triphosphates (NTPs), and detecting the incorporation of the NTP, is new. DETAILED DESCRIPTION - Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises: (a) hybridizing a detectable amount of a target nucleic acid polymer in single-stranded form with an oligonucleotide primer, where the detection primer comprises several nucleotide residues and is complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the primer is hybridized to the polymer, there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first or second nucleotide residues to be detected; (b) extending the primer using a polymerizing agent in a mixture containing one or more NTPs, and at least one NTP complementary to either the first or second nucleotide residue that comprises a means for detecting the incorporation of the NTP in a nucleic acid polymer, and optionally one or more chain terminating NTPs; and (c) detecting the incorporation of the NTP, where the identity of the nucleotide residue at the defined site is determined. INDEPENDENT CLAIMS are also included for: (1) detecting (M2) in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1; (2) a kit for determining the specific nucleotide variation in a target nucleic acid polymer comprising in a packaged combination of: (a) at least one amplification primer comprising an oligonucleotide complementary to and hybridizes with a portion of the target nucleic acid polymer and is effective as a primer for enzymatic nucleic acid polymerization and a first attachment moiety; (b) at least one detection step primer comprising an oligonucleotide complementary to and hybridizes with a portion 3' to a variable nucleotide of the target nucleic acid polymer; and optionally; (c) at least one solid support comprising a solid matrix and at least one attachment site which is capable of %%%immobilizing%%% the oligonucleotide of the amplification probe through the first attachment moiety; and (d) at least one NTP containing a means for detecting the incorporation of the NTP in a nucleic acid polymer; (3) a reagent for detecting the presence of a point mutation in which a normal nucleic acid residue is replaced by an abnormal nucleic acid residue at a defined site within gene of interest; (4) detecting at a defined site in the genome of a microorganism, the existence of point mutations leading to altered pathogenicity or resistance to therapy in microorganisms, where the first nucleotide residue is replaced by a second nucleotide residue, by obtaining a sample containing a detectable amount of genetic material obtained from the microorganism, and employing the steps of M1; and (5) detecting cells having a point mutation at a defined site in the genetic material, where the first nucleotide residue is replaced by a second nucleotide residue, and the mutated cells are mixed in a cell population with unmutated cells, by obtaining a detectable quantity of genetic material from the cell population while maintaining the ratio of mutated to unmutated cells, and employing the steps of M1. The cells are lymphocytes, where the mutated cells are leukemic cells.

BIOTECHNOLOGY - Preferred Method: M1 further comprise removing the extended detection step primer from the target nucleic acid polymer, and adding a second detection step primer complementary to the nucleotide sequence of interest in a region disposed toward the 3' end

from the second defined site such that when the primer is hybridized to the %%%immobilized%%% polymer, there are no nucleotide residues between the second defined site and the 3' end of the primer that are identical to the third or fourth nucleotide residues to be detected. Detecting in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1. M1 and M2 further comprise %%%immobilizing%%% the target nucleic acid polymer to a solid support before hybridizing the target nucleic acid to an oligonucleotide polymer. The primer is complementary to a region of the nucleotide sequence of interest extending toward the 3' end of the target nucleic acid polymer from the nucleotide residue immediately adjacent to the defined site. The NTP comprising a means for detecting the incorporation of the NTP in a nucleic acid polymer is a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate. The mixture includes a second NTP comprising a second means, different from the first, for detecting the incorporation of the second nucleotide triphosphate in a nucleic acid polymer. The extended product is eluted before determining the incorporation of the incorporated NTP. The nucleotide variations are detected in one single step by adding several detection primers and differently labeled NTPs identifying the variable nucleotide residues. The detectable amount of target nucleic acid polymer is obtained by performing a modified amplification reaction where at least one amplification primer comprises a first attachment moiety bonded to the primer. For the method of (4), the microorganism is human immunodeficiency virus, and the point of mutation is at a site selected from Asp67, Lys70 and Thr215. Preferred Kit: The kit for identifying nucleotide variation of %%%gapolipoprotein%%% E polymorphism comprises a detection step primer having a sequence selected from: (p1) 5'-GCG %%%CGG%%% ACA TGG AGG ACG TG; (p2) 5'-ATG CCG ATG ACC TGC AGA AG; (p3) 5'-GTA CTG CAC CAG GCG GCC GC; and (p4) 5'-GGC CTG GTA CAC TGC CAG GC. The kit for detecting nucleotide variation in codon 6 of the human beta-globin gene causing sickle cell anemia comprises a detection step primer having the sequence: (p5) 5'-CAT GGT GCA CCT GAC TCC TG; or (p6) 5'-CAG TAA %%%CGG%%% CAG GCG GCC GC. The kit for detecting a nucleotide variation in codon 12 of the K-ras gene comprises a detection step primer having a sequence selected from: (p7) 5'-AAG GCA CTC TTG CCT ACG CCA; (p8) 5'-AGG CAC TCT TGC CTA CGC CAG; (p9) 5'-AAC TTG TGG TAG TTG GAG CT; and (p10) 5'-ATC TGT GGT AGT TGG AGC TG. Preferred Reagent: The reagent comprises an oligonucleotide of sufficient length to act as a primer for an enzyme catalyzed chain extension nucleic acid polymerization reaction, where the oligonucleotide primer has a sequence which is complementary to a region of the gene of interest beginning with the nucleotide residue immediately adjacent to and toward the 3' end of the gene from the defined site and extending away from the defined site toward the 3' end of the gene, where an enzyme catalyzed chain extension nucleic acid polymerization will commence by adding a nucleic acid residue complementary to either the normal nucleic acid residue of the abnormal nucleic acid residue. The polynucleotide has a length of 10-40 nucleotide residues, and has a sequence selected from p1-p10. USE - M1 is useful for identifying specific point mutations and genetic variations. Specifically, the method can be used for pre- or postnatal diagnosis of hereditary predispositions or diseases, for the detection of somatic mutations in cancer, for the selection of cells and strains for industrial biotechnology and for plant and animal breeding. ADVANTAGE - The new method provides several advantages over prior methods. The new method comprises few and easily performed procedures, thus especially suited for routine determinations of point mutations and nucleotide variations, allows the quantification of the proportion of mutated cells in a sample as well as the identification of mutations present in as little as 0.5% of the analyzed cell population, and is easily automated. EXAMPLE - Four PCR primers (P1-P4) and 2 detection step oligonucleotide primers (D1 and D2) were synthesized. A biotinylated 5'-aminogroup was added to primer P2 with the aminolink II reagent. Leukocytic DNA was extracted from venous blood samples obtained from patients of known Apo E phenotype. DNA was amplified with P1 and P4

primers. An aliquot of the first PCR amplification mixture was transferred to a second PCR and directed by the biotinylated primers P2 and P3. The second amplification mixture was added with 5 μl of 5% suspension of avidin-coated polystyrene particles, kept for 20 degrees C for 30 minutes, collected by centrifugation, washed, and treated with NaOH. Suspension of particles was divided into 4 parts and collected by centrifugation in separate tubes. Particles carrying the DNA fragment were suspended in NaCl, MgCl<sub>2</sub>, Tris-HCl containing 2 pmol of the detection step primer. D1 oligonucleotide located immediately adjacent to the variable nucleotide 3745 was added to 2 of the tubes and D2 oligonucleotide adjacent to the variable nucleotide 3883 to 2 tubes. Oligonucleotide was annealed to the DNA template by heating the samples at 65 degrees C for 2 minutes, and cooling them to 20 degrees C. Dithiothreitol and (35S)-labeled deoxynucleoside triphosphates (dNTP) and dideoxynucleoside triphosphates (ddNTP) were added to yield 1 μM concentration of each final volume of 15 μl for identification of T: (35S)-dTTP, ddCTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed; and (35S)-dCTP, ddTTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed. T7 DNA polymerase was added to each tube and allowed the reaction to proceed. Products were eluted and radioactivity in liquid scintillation counter was measured. The differences in cpm values obtained in the T- and C-reactions allowed unequivocal identification of the variable nucleotide in both codons 112 and 118 in all 4 DNA samples. (16 pages)

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0320372 DBR Accession No.: 2003-21512 PATENT

Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, useful for pre- or postnatal diagnosis of diseases, comprises extending the detection primer using labeled nucleotide triphosphates - nucleotide variation detection for use in disease diagnosis and plant and animal breeding

AUTHOR: SODERLUND H E; SYVANEN A

PATENT ASSIGNEE: SODERLUND H E; SYVANEN A 2003

PATENT NUMBER: US 20030082531 PATENT DATE: 20030501 WPI ACCESSION NO.: 2003-596956 (200356)

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NATIONAL APPLIC. NO.: US 258216 APPLIC. DATE: 19990226

LANGUAGE: English

ABSTRACT: DERVENT ABSTRACT: NOVELTY - Detecting (M1) a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises extending the primer using a polymerizing agent in a mixture containing one or more nucleoside triphosphates (NTPs), and detecting the incorporation of the NTP. DETAILED DESCRIPTION - Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises: (a) hybridizing a detectable amount of a target nucleic acid polymer in single-stranded form with an oligonucleotide primer, where the detection primer comprises several nucleotide residues and is complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the primer is hybridized to the polymer, there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first or second nucleotide residues to be detected; (b) extending the primer using a polymerizing agent in a mixture containing one or more NTPs, and at least one NTP complementary to either the first or second nucleotide residue that comprises a means for detecting the incorporation of the NTP in a nucleic acid polymer, and optionally one or more chain terminating NTPs; and (c) detecting the incorporation of the NTP, where the identity of the nucleotide residue at the defined site is determined. INDEPENDENT CLAIMS are also included for the following: (1) detecting (M2) in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second

nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1; (2) a kit for determining the specific nucleotide variations in a target nucleic acid polymer comprising in a packaged combination: (a) at least one amplification primer comprising an oligonucleotide complementary to and hybridizes with a portion of the target nucleic acid polymer and is effective as a primer for enzymatic nucleic acid polymerization and a first attachment moiety; (b) at least one detection step primer comprising an oligonucleotide complementary to and hybridizes with a portion 3' to a variable nucleotide of the target nucleic acid polymer; and optionally; (c) at least one solid support comprising a solid matrix and at least one attachment site which is capable of %%%immobilizing%%% the oligonucleotide of the amplification probe through the first attachment moiety; and (d) at least one NTP containing a means for detecting the incorporation of the NTP in a nucleic acid polymer; (3) a reagent for detecting the presence of a point mutation in which a normal nucleic acid residue is replaced by an abnormal nucleic acid residue at a defined site within gene of interest; (4) detecting at a defined site in the genome of a microorganism, the existence of point mutations leading to altered pathogenicity or resistance to therapy in microorganisms, where the first nucleotide residue is replaced by a second nucleotide residue, by obtaining a sample containing a detectable amount of genetic material obtained from the microorganism, and employing the steps of M1; and (5) detecting cells having a point mutation at a defined site in the genetic material, where the first nucleotide residue is replaced by a second nucleotide residue, and the mutated cells are mixed in a cell population with unmutated cells, by obtaining a detectable quantity of genetic material from the cell population while maintaining the ratio of mutated to unmutated cells, and employing the steps of M1. The cells are lymphocytes, where the mutated cells are leukemic cells. BIOTECHNOLOGY - Preferred Method: M1 further comprise removing the extended detection step primer from the target nucleic acid polymer, and adding a second detection step primer complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the second defined site such that when the primer is hybridized to the %%%immobilizing%%% polymer, there are no nucleotide residues between the second defined site and the 3' end of the primer that are identical to the third or fourth nucleotide residues to be detected. Detecting in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1. M1 and M2 further comprise %%%immobilizing%%% the target nucleic acid polymer to a solid support before hybridizing the target nucleic acid to an oligonucleotide polymer. The primer is complementary to a region of the nucleotide sequence of interest extending toward the 3' end of the target nucleic acid polymer from the nucleotide residue immediately adjacent to the defined site. The NTP comprising a means for detecting the incorporation of the NTP in a nucleic acid polymer is a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate. The mixture includes a second NTP comprising a second means, different from the first, for detecting the incorporation of the second nucleotide triphosphate in a nucleic acid polymer. The extended product is eluted before determining the incorporation of the incorporated NTP. The nucleotide variations are detected in one single step by adding several detection primers and differently labeled NTPs identifying the variable nucleotide residues. The detectable amount of target nucleic acid polymer is obtained by performing a modified amplification reaction where at least one amplification primer comprises a first attachment moiety bonded to the primer. For the method of (4), the microorganism is human immunodeficiency virus, and the point of mutation is at a site selected from Asp67, Lys70 and Thr215. Preferred Kit: The kit for identifying nucleotide variation of %%%apolipoprotein%%% E polymorphism comprises a detection step primer having a sequence selected from: (p1) 5'-GCG %%%CGG%%% ACA TGG AGG ACG TG; (p2) 5'-ATG CCG ATG ACC TGC AGA AG; (p3) 5'-GTA CTG CAC CAG GCG GCC GC; and (p4) 5'-GGC CTG GTA CAC TGC CAG GC. The kit for detecting nucleotide variation in codon 6 of the

human beta-globin gene causing sickle cell anemia comprises a detection step primer having the sequence: (p5) 5'-CAT GGT GCA CCT GAC TCC TG; or (p6) 5'-CAG TAA %%%CGG%%% CAG GCG GCC GC. The kit for detecting a nucleotide variation in codon 12 of the K-ras gene comprises a detection step primer having a sequence selected from: (p7) 5'-AAG GCA CTC TTG CCT ACG CCA; (p8) 5'-AGG CAC TCT TGC CTA CGC CAG; (p9) 5'-AAC TTG TGG TAG TTG GAG CT; and (p10) 5'-ATC TGT GGT AGT TGG AGC TG. Preferred Reagent: The reagent comprises an oligonucleotide of sufficient length to act as a primer for an enzyme catalyzed chain extension nucleic acid polymerization reaction, where the oligonucleotide primer has a sequence which is complementary to a region of the gene of interest beginning with the nucleotide residue immediately adjacent to and toward the 3' end of the gene from the defined site and extending away from the defined site toward the 3' end of the gene, where an enzyme catalyzed chain extension nucleic acid polymerization will commence by adding a nucleic acid residue complementary to either the normal nucleic acid residue of the abnormal nucleic acid residue. The polynucleotide has a length of 10-40 nucleotide residues, and has a sequence selected from p1-p9. USE - The method is useful for identifying specific point mutations and genetic variations. Specifically, the method can be used for pre- or postnatal diagnosis of hereditary predispositions or diseases, for the detection of somatic mutations in cancer, for the selection of cells and strains for industrial biotechnology and for plant and animal breeding.

**ADVANTAGE** - The new method provides several advantages over prior methods. The new method comprises few and easily performed procedures, thus especially suited for routine determinations of point mutations and nucleotide variations, allows the quantification of the proportion of mutated cells in a sample as well as the identification of mutations present in as little as 0.5% of the analyzed cell population, and is easily automated.

**EXAMPLE** - Four PCR primers (P1-P4) and 2 detection step oligonucleotide primers (D1 and D2) were synthesized. A biotinylated 5'-amino group was added to primer P2 with the aminolink II reagent. Leukocytic DNA was extracted from venous blood samples obtained from patients of known Apo E phenotype. DNA was amplified with P1 and P4 primers. An aliquot of the first PCR amplification mixture was transferred to a second PCR and directed by the biotinylated primers P2 and P3. The second amplification mixture was added with 5 microl of 5% suspension of avidin-coated polystyrene particles, kept for 20degreesC for 30 minutes, collected by centrifugation, washed, and treated with NaOH. Suspension of particles was divided into 4 parts and collected by centrifugation in separate tubes. Particles carrying the DNA fragment were suspended in NaCl, MgCl<sub>2</sub>, Tris-HCl containing 2 pmol of the detection step primer. D1 oligonucleotide located immediately adjacent to the variable nucleotide 3745 was added to 2 of the tubes and D2 oligonucleotide adjacent to the variable nucleotide 3883 to 2 tubes. Oligonucleotide was annealed to the DNA template by heating the samples at 65degreesC for 2 minutes, and cooling them to 20degreesC. Dithiothreitol and (35S)-labeled deoxynucleoside triphosphates (dNTP) and dideoxynucleoside triphosphates (ddNTP) were added to yield 1 microM concentration of each final volume of 15 microl for identification of T: (35S)-dTTP, ddCTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed; and (35S)-dCTP, ddTTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed. T7 DNA polymerase was added to each tube and allowed the reaction to proceed. Products were eluted and radioactivity in liquid scintillation counter was measured. The differences in cpm values obtained in the T- and C-reactions allowed unequivocal identification of the variable nucleotide in both codons 112 and 118 in all 4 DNA samples. (16 pages)

2/7/8 (Item 6 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.  
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0288714 DBR Accession No.: 2002-10561 PATENT

Determining mismatch binding %%%protein%%% in sample comprises mixing sample with labeled heteroduplex DNA, contacting mixture with %%%immobilized%%% mismatch binding %%%protein%%% and detecting amount

of DNA bound and comparing with control - streptavidin and horseradish peroxidase conjugate for fragile-X syndrome, myotonic dystrophy, Huntington chorea, spino-cerebellar ataxia type-I, spinal bulbar muscular atrophy, Machado-Joseph disease and dentatorubralpallidoluysian atrophy diagnosis

AUTHOR: WAGNER R E

PATENT ASSIGNEE: VALIGEN US INC 2001

PATENT NUMBER: US 6329147 PATENT DATE: 20011211 WPI ACCESSION NO.: 2002-187350 (200224)

PRIORITY APPLIC. NO.: US 497933 APPLIC. DATE: 20000204

NATIONAL APPLIC. NO.: US 497933 APPLIC. DATE: 20000204

LANGUAGE: English

**ABSTRACT:** DERWENT ABSTRACT: NOVELTY - Determining (M1) the presence of a functional mismatch binding %%%protein%%% comprising mixing biological fluid sample (FS) with detectably labeled heteroduplex DNA (I) to form a mixture, contacting the mixture with %%%immobilized%%% mismatch-binding %%%protein%%% (II), detecting the binding of (I) to (II) and comparing the amount of (I) bound with the amount of (I) bound when control sample (CS) is utilized instead of FS, is new. DETAILED DESCRIPTION - Determining (M1) the presence of a functional (II) in a biological fluid sample comprising: (a) mixing FS with (I) to form a mixture; (b) contacting the mixture with an %%%immobilized%%% (II); (c) detecting the binding of (I) to (II); and (d) comparing the amount of (I) bound with an amount bound when steps (a)-(c) are separately performed using a CS in the place of FS, where a reduced amount of bound (I) in the presence of FS relative to CS is indicative of presence of functional (II) in FS. An INDEPENDENT CLAIM is also included for detecting (M2) the presence of a triplet repeat block of unit sequence. BIOTECHNOLOGY - Preferred Method: In M1, the %%%immobilized%%% (II) is preferably Escherichia coli MutS %%%protein%%% or their homolog from a different prokaryotic or eukaryotic species. M2 comprises: (a) determining an unit repeat sequence of the repeat block in the test DNA, where the repeat sequence but not its complement forms secondary structure which binds to an %%%immobilized%%% (II); (b) incubating (a) test DNA with (II) which is a detectably labeled single stranded diagnostic oligonucleotide probe having a unit triplet sequence 3'-(GCC)m or 3'-(GAC)m (or 3'-(%%%CGG%%% )m or 3'-(CTG)m), where m is an integer, and is number of repeats of TRB of the probe; (c) denaturing double stranded DNA in the mixture into single strands and allowing the strands to reanneal; (d) incubating the mixture obtained with an %%%immobilized%%% (II); and (e) detecting the binding of detectably labeled DNA to the (II), where the presence of bound detectably labeled DNA is indicative of the presence of the test DNA of TRB with a repeat number n greater than m so as to detect the presence of a TRB of unit sequence in test DNA in a sample, and where the test DNA is obtained from an individual at a risk for developing or transmitting a disease or syndrome associated with triplet repeats, which is preferably from fragile X syndrome type A, fragile X syndrome type E, myotonic dystrophy, Huntington's disease, spino-cerebellar ataxia type I, spinal bulbar muscular atrophy, Machado-Joseph disease and dentatorubralpallidoluysian atrophy. The method further comprises amplifying test DNA prior to step (a). USE - M1 and M2 are useful for determining the presence of a functional (II) in a biological fluid sample preferably presence of a repeat block especially a triplet repeat block (TRB) of unit sequence 5'-(%%%CGG%%% )n or 5'-(CTG)n 3'-(GCC)n 3'-(GAC)n in test DNA in a sample preferably by a competitive assay method, where TRB is longer (or shorter) than TRB in a diagnostic oligonucleotide probe, and n is an integer and is number of repeats of TRB in the test DNA (claimed). The method is useful for diagnosing a variety of disease states or susceptibilities such as fragile X syndrome, myotonic dystrophy, Huntington's disease, spino-cerebellar ataxia type I, spinal bulbar muscular atrophy, Machado-Joseph disease and dentatorubralpallidoluysian atrophy. EXAMPLE - Escherichia coli MutS (mismatch binding %%%protein%%%; MBP) was purified and spotted on nitrocellulose paper in each well, and the nitrocellulose filter obtained was blocked with bovine serum albumin (BSA) to prevent binding of other %%%proteins%%% or nucleic acids. The sequence of oligonucleotides was taken from 30 base region surrounding the site of sickle cell mutation in the human J-globin gene, where the mismatch was at the site of the mutation, although the mutant sequence

used to form the mismatch is not the mutation, and biotinylated oligonucleotides were biotinylated on the 5' end of the mutant strand. Then, the biotinylated oligonucleotides were added to each well, and the presence of the biotin was detected by its binding of streptavidin, which comprised adding streptavidin-conjugated horse radish-peroxidase (HRP). Further the nitrocellulose sheet was removed from dot blot apparatus and washed with reaction buffer, and Enhanced Chemiluminescence (ECL) was poured over the nitrocellulose, where ECL comprised the substrate for HRP. After 1 minute the solution was removed and the nitrocellulose was blotted dry and placed between clear plastic sheets, then was exposed to X-ray film in dark for varying periods of time. In competition studies, a constant amount of biotinylated mismatch-containing oligonucleotide was mixed with varying amounts of unlabeled DNA, with or without mismatch and added to the wells. Results showed that the %%immobilized%% MBP detected as little as 0.2 ng of mismatch containing 30-mer, whereas no detectable binding of mismatch-free 30-mer was observed even with 200 ng of DNA. Results of competition study showed that competition was clearly visible with 50 ng of mismatch containing DNA, and the mismatch-free DNA did not compete until 500 ng. Thus the results indicate %%immobilized%% discriminates between the mismatch containing and perfectly paired DNA with an efficiency of at least three orders of magnitude. Similar results have been obtained using 54-mers with a sequence derived from the V3 loop of HIV. Therefore, even if the discrimination decreases as the amount of perfectly paired duplex increases, the discrimination efficiency when using 300-mers, considered to be the maximum useful length for polymorphism studies of the human genome, should be on the order of a factor of 100. (62 pages)

2/7/9 (Item 7 from file: 357)  
DIALOG(R)File 357:Dowd Biotech Res.  
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0285278 DBR Accession No.: 2002-07125 PATENT

Detecting growth hormone variants (GH1), useful in screening patients for growth hormone irregularities, comprises comparing the nucleotide sequence of a GH1 gene from a test sample with that of a standard sequence of the human GH1 - vector-mediated recombinant somatotropin gene transfer and expression in bacterium, polymerase chain reaction, DNA primer, drug screening and DNA microarray for disease diagnosis and gene therapy

AUTHOR: COOPER D N; PROCTER A M; GREGORY J; MILLAR D S

PATENT ASSIGNEE: UNIV WALES COLLEGE OF MEDICINE 2001

PATENT NUMBER: WO 200185993 PATENT DATE: 20011115 WPI ACCESSION NO.: 2002-089798 (200212)

PRIORITY APPLIC. NO.: EP 2000306004 APPLIC. DATE: 20000714

NATIONAL APPLIC. NO.: WO 2001GB2126 APPLIC. DATE: 20010514

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Detecting a variation in GH1 effective as an indicator of GH dysfunction in an individual comprises comparing the nucleotide sequence of GH1 gene obtained from the test sample with a standard sequence known to be that of the human GH1 gene, where a difference between the test sample and the standard sequence indicates the presence of a variation (GH1 variant), is new. DETAILED DESCRIPTION - The sample is obtained from an individual exhibiting growth failure, defined as a growth pattern delineated by a series of height measurements, Brook CDG, Clinical Pediatric Endocrinology, 3rd Ed:141, 1995), which when plotted on a standard height chart (Tanner, et al., Arch Dis Child. 45:755-762, 1970), predicts an adult height for the individual which is outside the individual's estimated target adult height range, where the estimate is based upon the heights of the individual's parents. INDEPENDENT CLAIMS are also included for the following: (1) GH1 variants; (2) a %%protein%% or amino acid sequence encoded by a GH1 variant; (3) human GH variants; (4) methods of screening an individual suspected of GH dysfunction; (5) kits for carrying out the screening methods comprising: (a) an oligonucleotide having a sequence corresponding to a region of a GH1 variant, where the region incorporates at least one variation from the corresponding wild type hGH gene sequence; and/or (b) an oligonucleotide having a sequence

corresponding to the wild type hGH gene sequence in the region specified in (a); and optionally (c) one or more reagents for carrying out PCR for amplifying desired regions of the individual's DNA; (6) an isolated, purified or recombinant nucleic acid sequence selected from: (a) a sequence comprising a GH1 variant or encoding a GH1 variant as defined above; (b) a sequence substantially homologous to or that hybridizes to (a) under stringent conditions; (c) a sequence substantially homologous to or that hybridizes to, under stringent conditions, to (a) or (b) but for the degeneracy of the genetic code; or (d) an oligonucleotide specific for (a), (b) or (c); (7) a vector comprising the nucleic acid; (8) a host cell comprising the vector, such as a bacterial host cell; (9) a process for preparing a GH1 variant defined above; (10) an amino acid sequence encoded or expressed by a sequence, vector, or cell in culture medium; and (11) a composition comprising a GH1 or GH variant and a carrier. BIOTECHNOLOGY - Preferred Method: The test sample is obtained from an individual exhibiting at least one of the following further criteria: height velocity below the 25th centile for age, and/or bone age delay according to the Tanner-Whitehouse scale of at least 2 years when compared with chronological age, and/or no other disorder known to cause inclusion in the criteria mentioned. Bone age delay is 2-4 years when compared with chronological age. The individual exhibits normal results in a standard growth hormone function test. The detection method comprises sequencing method for determining the sequence of the GH1 gene of an individual. The detection method comprises PCR amplification of the GH1 gene of the individual using a GH1 gene-specific fragment which is a fragment unique to the GH1 gene whose sequence is not found in 4 other paralogous (non-GH1) genes in the GH cluster, and one or more GH1 gene-specific primers which cannot bind to the homologous flanking regions in the 4 other paralogous genes in the GH cluster. Alternatively, the detection method comprises PCR amplification of the entire GH1 gene of the individual and nested PCR of overlapping constituent fragments of the GH1 gene of the individual, PCR amplification of all or a fragment of genomic DNA spanning the Locus Control Region of the GH1 gene, or mutational screening of all or a fragment of the individual's GH1 gene by DPLC. The detection method may comprise the use of one or more primers selected from 29 sequences, e.g. CTG CGC GTT CAG GTT GGC (GH1DF); AGG TGA GCT GTC CAC AGG (GH1DR); GGG CAA CAG TGG GAG AGA AG (GH2DF); CCT CCA GGG ACC AGG AGC (GH2DR); CAT GTA AGC CCA GTA TTT GGC C (GH3DF); CTG AGC TCC TTA GTC TCC TCC TCT (GH3DR); GAC TTT CCC CCG CTG GGA AA (GH4DF); GGA GAA GGC ATC CAC TCA %%CGG%% (GH4DR); TCA GAG TCT ATT CCG ACA CCC (GH5DF); and GTG TTT CTC TAA CAC AGC TCT C (GH5DR). Screening an individual suspected of GH dysfunction comprises comparing a region of an obtained nucleotide sequence of the human GH1 gene from the individual, with the corresponding region of a predetermined sequence, where the predetermined sequence is selected from a GH1 variant defined below, where the test sample comprises genomic DNA. Alternatively, the method comprises analyzing the test sample for the presence of a GH1 or GH variant, or for the presence of one or more surrogate markers that indicate or correlate to the presence of a GH1 or GH variant, which exhibits at least one variation when compared to the wild type hGH sequence. Simultaneous screens are used either for multiple known mutations or for all possible mutations by hybridization of a labeled sample of DNA (cDNA or genomic DNA derived from the individual) to micro-arrays of mutation-specific oligonucleotide probes %%immobilized%% on a solid support. A chip technology is used where the chip is a miniature parallel analytical device. Analyzing the sample for the presence of a GH variant includes conventional sequencing methods (such as mass spectroscopy, micro-array analysis or pyrosequencing), and/or antibody-based methods of detection (e.g. ELISA). A GH1 variant can be prepared by culturing a host cell comprising a vector defined above and recovering from the culture medium the variant GH1 produced. Preferred GH1 Variant: The GH1 variant is detected or is detectable by the method defined above, but is not detected by methods such as those reliant on patient selection criteria based primarily on absolute height. The variant may be selected from those characterized as unpublished in the specification Growth Hormone deficiency; GH1 gene mutations and polymorphisms, given in the specification. The variant comprises a missense or a silent mutation

which affects the activity of the signal %%%peptide%%% . The GH1 variant may comprise one or more GH1 promoter mutations selected from A to G -248, T to C -495, A to G -177, T to C - 30 (TATA), A to G - 24, C to T -347, A to G -44, A to G +62, G to A -48, A to G -498, T to C -508, deltaGGGGG -57 to -61, and approximately -57. The human GH variant comprises amino acid substitutions with respect to wild type GH selected from: M to V -26; T to A -20; L to P -11; F to L 1; I to V 4; D to N 11; Q to R 22; D to V 261 E to G 30; K to R 411 S to L 431 E to G 56; R to G 64; S to F 71; E to K 74; S to P 85; W to R 86; Q to L 91; D to G 107; S to C 108; S to R 108; V to I 110; Y to H 143; A to V 155; L to P 163; K to R 168; K to E 168; T to A 175; and F to S 176. The human GH variant may also be selected from one or more of the following (the locus on hGH are in the parentheses): Ile4Val (N-terminal, within site 2); Gln22Arg (helix 1); Lys41Arg (loop 1); Glu56Gly (in loop region between helices 1 and 2, part of binding site 1); Arg64Gly (loop 2); Lys168Arg (helix 4); Lys168Glu; and Thr175Ala (helix 4), as defined with respect to wild type GH. The human variant comprises the amino acid substitution with respect to wild type hGH: Glu to Gly 30, as shown in the specification. Preferred Kit: The kit comprises at least one of the variants defined above, and further several oligonucleotides on a solid support. ACTIVITY - Osteopathic. MECHANISM OF ACTION - Gene therapy; %%%protein%%% therapy. USE - The method is useful in screening patients for growth hormone irregularities or for producing variant %%%proteins%%% for treating such irregularities, and for the early detection and appropriate clinical management of familial GH deficiency. The GH1 variants are useful in therapeutic, diagnostic or detection method, particularly for determining binding defects and susceptibility to a disease such as diabetes, obesity or infection; for treating acromegaly or gigantism conditions associated with lactogenic, diabetogenic, lipolytic and %%%protein%%% anabolic effects, conditions associated with sodium and water retention, metabolic syndromes, mood and sleep disorders; and diagnosing GH dysfunction. The GH1 variants are especially useful in gene therapy or %%%protein%%% therapy. The GH1 or GH variant may also be used in the preparation of a medicament, diagnostics composition or kit, or detection kit (all claimed). ADVANTAGE - The method has the following advantages: (i) expansion of the known spectrum of GH1 gene mutations by identification and characterization of new lesions; (ii) evaluation of the role of GH1 gene mutations in the etiology of short stature; (iii) identification of the mode of inheritance of novel GH1 gene lesions; (iv) elucidation of the relationship between mutant genotype and clinical phenotype, which is deemed essential for the early detection and appropriate clinical management of GH deficiency; (v) evaluation of the effects of GH1 mutations on the structure and function of the GH molecule, which is important for the assessment of those children with clinical phenotype at the milder end of the clinical spectrum of short stature; (vi) development of rapid diagnostic tests for inherited GH deficiency; and (vii) assessment of the postulate that GH deficiency is currently under-diagnosed and underestimated in the population. EXAMPLE - No relevant example given. (95 pages)

2/7/10 (Item 8 from file: 357)  
DIALOG(R)File 357:Dowent Biotech Res.  
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0082133 DBR Accession No.: 89-00124 PATENT  
Insulin-binding %%%peptide%%% , monoclonal antibody and DNA or RNA probe production - useful for determining insulin concentrations and insulin receptor number or reducing insulin concentration in fluid; hybridoma construction

PATENT ASSIGNEE: Univ.Texas-Syst. 1988

PATENT NUMBER: EP 286147 PATENT DATE: 881012 WPI ACCESSION NO.: 88-287345 (8841)

PRIORITY APPLIC. NO.: US 36215 APPLIC. DATE: 870409

NATIONAL APPLIC. NO.: EP 88105737 APPLIC. DATE: 880411

LANGUAGE: English

ABSTRACT: Insulin-binding %%%peptides%%% of formula Y-Cys(Gly-Ser-Arg-Trp)-Val(Ile-Leu-Met)-Glu(Lys)-Glu(Lys)-Ala-(Pro-Ser-Thr)-Ser(Ala-Thr-Pro-X); Y-Cys(Gly-Ser-Arg)-Val(Ile)-Glu(Lys)-Glu(Lys)-Ala(Thr-Ser-Pro)

- Ser(Ala-Thr-Pro)-X; and Y-Cys(Trp)-Ile(Met)-Lys-Lys-Pro-Ala(Ser)-X (where each residue in brackets may be substituted by the preceding amino acid and X and Y are terminal or carboxyl amino groups, or a non-interfering fragment of up to 10 residues), polyclonal and monoclonal antibodies (MAbs) to the %%%peptides%%% and DNA and RNA probes for the insulin-binding %%%peptides%%% are new. The probes are preferably insulin B-chain (22-27)-Arg-Gly-Phe- Phe-Tyr-Thr- cDNA-CGA-GGC-TTC-TTC- TAC-ACA-(5'-3'); cDNA-ACA-CAT-CTT-CTT- %%%CGG%%% -AGC-(3'-5'); and mRNA-UGU-GUC-GAA-GAA -GCC-UCG-(5'-3'). Also new are methods of: (1) determining the concentration of insulin in a biological fluid which comprises using %%%immobilized%%% %%%peptides%%% , DNA probes, RNA probes or MAbs; and (2) reducing the concentration of insulin. The MAbs are preferably prepared by fusing cells of a mammal, injected with an epitope of a new insulin-binding %%%peptide%%% , with myeloma cells. (19pp)

2/7/11 (Item 1 from file: 370)  
DIALOG(R)File 370:Science  
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00501407 (USE 9 FOR FULLTEXT)  
Severe Fibronectin-Deposit Renal Glomerular Disease in Mice Lacking Uteroglobin  
Zhang, Zhongjian; Kundu, Gopal C.; Yuan, Chiun-Jye; Ward, Jerrold M.; Lee, Eric J.; DeMayo, Francesco; Westphal, Heiner; Mukherjee, Anil B.  
Z. Zhang, G. C. Kundu, C.-J. Yuan, A. B. Mukherjee, Section on Developmental Genetics, Heritable Disorders Branch, National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD 20892-1830, USA. ; J. M. Ward, Veterinary and Tumor Pathology Section, Office of Laboratory Animal Science, National Cancer Institute, Frederick, MD 21702-1201, USA. ; E. J. Lee and H. Westphal, Laboratory of Mammalian Genetics and Development, NICHD, NIH, Bethesda, MD 20892-1830, USA. ; F. DeMayo, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, USA.  
Science Vol. 276 5317 pp. 1408  
Publication Date: 5-30-1997 (970530) Publication Year: 1997  
Document Type: Journal ISSN: 0036-8075  
Language: English  
Section Heading: Reports  
Word Count: 3092  
Abstract: Despite myriads of biological activities ascribed to uteroglobin (UG), a steroid-inducible secreted %%%protein%%% , its physiological functions are unknown. Mice in which the uteroglobin gene was disrupted had severe renal disease that was associated with massive glomerular deposition of predominantly multimeric fibronectin (Fn). The molecular mechanism that normally prevents Fn deposition appears to involve high-affinity binding of UG with Fn to form Fn-UG heteromers that counteract Fn self-aggregation, which is required for abnormal tissue deposition. Thus, UG is essential for maintaining normal renal function in mice, which raises the possibility that an analogous pathogenic mechanism may underlie genetic Fn-deposit human glomerular disease.

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17. An experimental protocol was approved by the institutional animal care and use committee. Total RNAs were isolated from different organs of UG.sup(+/+), UG.sup(+/-), and UG.sup(-/-) mice and reverse transcribed with mouse UG-specific primer, mPr (5' prime) -ATC TTG CTT ACA CAG AGG ACT TG-3 (prime) . The PCR product was further amplified with primers mPI (5' prime) -ATC GCC ATC ACA ATC ACT GT-3 (prime) and mPr. The PCR product was hybridized with an oligonucleotide probe, mPp (5' prime) -ATC AGA GTC TGG TTA TGT GGC ATC C-3 (prime) , derived from exon-2 of the UG gene sequence. The primers and the probe used in mouse glyceraldehyde phosphate dehydrogenase (GAPDH) RT-PCR are as follows: mGAPDH-r (5' prime)-GGC ATC GAA GGT GGA AGA GT-3 (prime) ; mGAPDH-I (5' prime) -ATG GCC TTC CGT GTT CCT AC-3 (prime) ; and mGAPDH-p (5' prime)-GAA GGT GGT GAA GCA GGC ATC TGA GG-3 (prime) .;
18. Tissue lysates from the kidneys, liver, and lungs of UG.sup(+/+) and UG.sup(-/-) mice were prepared by homogenizing tissue samples in a buffer (10 mM tris-HCl, pH 7.5, 1% Triton X-100, 0.2% deoxycholate, 150 mM NaCl, 5 mM EDTA) containing 2 mM phenylmethylsulfonyl fluoride and 20 (mu) g/ml each of aprotinin, leupeptin, and pepstatin A. The homogenates were centrifuged at 17,500g for 30 min at 4.sup(o)C and immunoprecipitated as described [E. Harlow and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 1, 1988)] by incubating tissue lysates or plasma %%%proteins%%% (1 mg/ml) with rabbit antibody to murine Fn (anti-Fn) (1:100 dilution). Coimmunoprecipitation of purified murine Fn and recombinant human UG [G. Mantle et al., *J. Biol. Chem.*, 267, 20343 (1993)] was performed by incubating equimolar concentrations of Fn with UG in the presence of 10% glycerol, 50 mM tris-HCl, pH 7.5, 250 mM NaCl, and 4.3 mM sodium phosphate at 4.sup(o)C for 1 hour, followed by the addition of anti-Fn (1:100 dilution). Equal amounts of extracted tissue %%%proteins%%% (30 (mu) g) or immunoprecipitates were resolved either on 4 to 20% gradient or 6% SDS-polyacrylamide gels under reducing conditions, followed by %%%protein%%% immunoblotting with either anti-Fn (1:2000 dilution) or rabbit antibodies to murine UG (anti-UG) (1:2000 dilution). ;
19. Tissues from UG.sup(-/-), UG.sup(+/-), and UG.sup(+/+) mice were fixed in Bouin's fluid or in 10% neutral-buffered formalin fixatives, embedded in paraffin, and sectioned at 4 to 6 (mu) m. They were stained with hematoxylin and eosin (H and E). Selected tissues were stained by Masson's trichrome method for collagen detection, PTAH for fibrin, or Congo Red for amyloid %%%protein%%%%. For immunohistochemical detection of UG and Fn, the Vectastain rabbit Elite ABC kit (Vector Laboratories) was used. The rabbit antibody (CytImmune) to murine UG (mUG) was raised by using a synthetic %%%peptide%%% (%%%Peptide%%% Technologies) corresponding to mUG amino acid sequence Lys.sup(30) to Thr.sup(51). The rabbit antibody to murine Fn (Gibco-BRL) was used at a dilution of 1:1000, and the antibody to UG was used at 1:500. ;
20. A kidney from a UG.sup(-/-) mouse, with glomerular lesions, was fixed in formalin and embedded in epoxy resin. Thin sections stained with uranyl acetate and lead citrate were examined with an electron microscope. Photomicrographs were taken either at x 6000 or at x 60,000. ;
21. Formalin-fixed tissue sections were used for immunofluorescence as described (B5) with anti-Fn and fluorescein isothiocyanate (FITC)-conjugated goat antibody to rabbit IgG. Similarly, immunofluorescence studies with antibodies specific for Fn, collagens I and III, vitronectin, laminin, and osteopontin were also done. Epifluorescence was photographed with a Zeiss Axiphot microscope. ;
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24. Age-, sex-, and weight-matched UG.sup(+/+) (n = 3) and UG.sup(-/-) mice (n = 3) were killed and serum PLA.inf(2) activities of each sample were measured in triplicate with a PLA.inf(2)-assay kit (Caymen Chemical) according to the instructions of the manufacturer. %%%Protein%%% concentrations in the sera were determined by Bradford assay (Bio-Rad), and specific activities of PLA.inf(2) were determined. ;
25. Using 24-well plates coated with human Fn (hFn) (Collaborative Biomedical Products), we incubated 3 (mu) l of .sup(125)I-Fn (specific activity of 6 (mu) Ci/ (mu) g; ICN Biomedicals) in the absence and presence of either UG or Fn (10.sup(-12) to 10.sup(-6) M) in 500 (mu) l of Hanks' balanced salt solution (HBSS) at room temperature for 2 hours. SDS-PAGE and %%%protein%%% immunoblotting of all Fn with anti-UG failed to detect any UG contamination. The radiolabeled complex was washed twice with phosphate-buffered saline (PBS), solubilized in 1 N NaOH, and neutralized with 1 N HCl, and radioactivity was measured by a gamma counter. In a separate experiment .sup(125)I-hFn (3 (mu) l) was incubated with 20 (mu) l (1 (mu) g/ (mu) l) of mouse Fn in 40 (mu) l of HBSS, pH 7.6, in the absence or presence of increasing concentrations of reduced UG (5 to 500 (mu) g) at room temperature for 2 hours. The samples were cross-linked with 0.20 mM DSS at room temperature for 20 min, boiled in SDS sample buffer for 5 min, electrophoresed on a 4 to 20% gradient SDS-polyacrylamide gel, and autoradiographed. In another experiment, 15 (mu) l of either denatured or nondenatured .sup(125)I-collagen I (specific activity of 65.4 (mu) Ci/ (mu) g) was incubated with Fn in presence of reduced UG (250 (mu) g), affinity cross-linked, electrophoresed, and autoradiographed. ;
26. Human Fn (500 (mu) g/150 (mu) l of PBS) was administered in the tail vein (B27) of 2-month-old, ~22-g, UG.sup(+/+) and apparently healthy UG.sup(-/-) mice. Similarly, the control mice were injected with a mixture of 500 (mu) g of hFn either with equimolar concentrations of UG or BSA in 150 (mu) l of PBS. Twenty-four hours after the last injection, the mice were killed and various organs fixed in buffered formalin. Accumulation of hFn immunoreactivity in tissues was analyzed by immunofluorescence (B6) with a monoclonal antibody to hFn (Gibco-BRL; clone 1) and FITC-conjugated rabbit antibody to mouse IgG (Cappel). In a separate experiment, UG.sup(+/+) mice were injected with 1 mg of Fn alone in 150 (mu) l of PBS daily for three consecutive days. ;
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31. A Bam HI-Eco RI 3.2-kb DNA fragment containing exon-3 of 129/SV mouse UG gene (B14) and its flanking sequences were subcloned into the corresponding site of the pPNW vector as described [K. Lei et al., *Nature Genet.* 13, 203 (1996)], A 0.9-kb PCR-amplified fragment containing

partial exon-2 and its flanking sequences with built-in Not I and Xho I sites were subcloned into the vector to generate the targeted construct pPNWUG. In pPNWUG, a 1.2-kb DNA fragment, including partial exon-2, was replaced with the PGK-neo cassette, disrupting the UG gene. Not I-linearized targeting construct DNA (25 ( $\mu$ g) was electroporated into ES R1 cells. Chimeric mice were mated with C57BL/6 strain of mice, and germline transmission of the mutated UG allele was identified by PCR and Southern blot analyses of offspring tail DNA. The genotyping of progeny was carried out by PCR with a set of neo-specific primers, neo-L (5' (prime) -ATA CGC TTG ATC %%CGG%% CTA CCT GCC-3 (prime)) and neo-R (5' (prime) -CAT TTG CAC TGC %%CGG%% TAG AAC TCC-3 (prime)), which yield a 667-base pair (bp) DNA fragment. A 304-bp DNA fragment was generated with a set of UG-specific primers, mUG-L (5' (prime) -ACA TCA TGA AGC TCA CAG GTA TGC-3 (prime)) and mUG-R (5' (prime) -GTG TGC ACG GTT CAA GCT TGT AGT-3 (prime)), derived from the region of the UG gene that was replaced by the PGK-neo cassette. After an initial denaturing step (95.Deg.C for 2 min), 40 cycles of PCR were performed (94.Deg.C for 1 min, 58.Deg.C for 1.5 min, 72.Deg.C for 1 min) with a final step at 72.Deg.C for 10 min, by using a Perkin-Elmer 480 DNA thermal cycler.;  
 32. The contributions of G.C.K. and C.-J.Y. in delineating the mechanism of UG action should be considered equal. We thank A. Nagy, R. Nagy, and W. Abramow-Newerly for ES R1 cells; L. Miele for statistical analyses; L. Miele and G. Mantle-Selvaggi for facilitating recombinant UG production in Escherichia coli; A. Kulkarni, K. M. Yamada, J. Chou, I. Owens, S. W. Levin, J. DeB. Butler, K.-J. Lei, and C.-J. Pan for their assistance, discussions, and suggestions, and Syntex Research for gancyclovir. Supported in part by a USPHS grant (number HL47620 to F.D.).

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DIALINDEX(R)  
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\*\*\* DIALINDEX search results display in an abbreviated \*\*\*  
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 3 135: NewsRx Weekly Reports\_1995-2009/Jan W1  
 4 357: Derwent Biotech Res.\_1982-2008/Nov W5  
 2 399: CA SEARCH(R)\_1967-2009/UD=15005

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10507336 Genuine Article#: 536FJ Number of References: 37  
 Title: A technique to %%/immobilize%% bioactive proteins, including bone

morphogenetic protein-4 (BMP-4), on titanium alloy

Author(s): Puleo DA (REPRINT); Kissling RA; Sheu MS

Corporate Source: Univ Kentucky,Ctr Biomed Engn, Wenner Gren Lab 205,Lexington//KY/40505 (REPRINT); Univ Kentucky,Ctr Biomed Engn, Wenner Gren Lab 205,Lexington//KY/40505; AST Prod,Billerica/MA/01821

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Abstract: %%/Immobilization%% of biomolecules on surfaces enables both localization and retention of molecules at the cell-biomaterial interface. Since metallic biomaterials used for orthopedic and dental implants possess a paucity of reactive functional groups, biomolecular modification of these materials is challenging. In the present work, we investigated the use of a plasma surface modification strategy to enable %%/immobilization%% of bioactive molecules on a "bioinert" metal. Conditions during plasma polymerization of allyl amine on Ti-6Al-4V were varied to yield 5 ("low")- and 12 ("high")-NH2/nm<sup>2</sup>. One- and two-step carbodiimide schemes were used to %%/immobilize%%

lysozyme, a model biomolecule, and bone morphogenetic protein-4 (BMP-4) on the aminated surfaces. Both schemes could be varied to control the amount of protein bound, but the one-step method destroyed the activity of %%%immobilized%%% lysozyme because of crosslinking. BMP-4 was then %%%immobilized%%% using the two-step scheme. Although BMP bound to both low- and high-NH<sub>2</sub> surfaces was initially able to induce alkaline phosphatase activity in pluripotent C3H10T1/2 cells, only high amino group surfaces were effective following removal of weakly bound protein by incubation in cell culture medium. (C) 2002 Elsevier Science Ltd. All rights reserved.

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DIALOG(R)File 135:NewsRx Weekly Reports  
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0000528817 (THIS IS THE FULLTEXT)  
University of Brescia, Italy, researchers describe recent findings  
Pharma Business Week, May 21, 2007, p.2776

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English  
RECORD TYPE: FULLTEXT  
AUDIENCE: Professional  
WORD COUNT: 1022

TEXT: University of Brescia, Italy, researchers describe recent findings. This trend article about University of Brescia, Italy, is an immediate alert from NewsRx to identify developing directions of research. Study 1: Fresh data on angiogenesis are presented in the report "Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor. Angiogenesis plays a key role in various physiologic and pathologic conditions, including tumor growth. Drm/gremlin, a member the Dan family of bone morphogenic protein (BMP) antagonists, is commonly thought to affect different processes during growth, differentiation, and development by heterodimerizing various BMPs," scientists in Italy report. "Here, we identify Drm/gremlin as a novel proangiogenic factor expressed by endothelium. Indeed, Drm/gremlin was purified to homogeneity from the conditioned medium of transformed endothelial cells using an endothelial-cell sprouting assay to follow protein isolation. Accordingly, recombinant Drm/gremlin stimulates endothelial-cell migration and invasion in fibrin and collagen gels, binds with high affinity to various endothelial cell types, and triggers tyrosine phosphorylation of intracellular signaling proteins. Also, Drm/gremlin induces neovascularization in the chick embryo chorioallantoic membrane. %%%BMP4%%% does not affect Drm/gremlin interaction with endothelium, and both molecules exert a proangiogenic activity in vitro and in vivo when administered alone or in combination. Finally, Drm/gremlin is produced by the stroma of human tumor xenografts in nude mice, and it is highly expressed in endothelial cells of human lung tumor vasculature when compared with non-neoplastic lung," wrote H. Stabile and colleagues, University of Brescia. The researchers concluded: "Our observations point to a novel, previously unrecognized capacity of Drm/gremlin to interact directly with target endothelial cells and to modulate angiogenesis." Stabile and colleagues published their study in Blood (Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor. Blood, 2007;109(5):1834-40). For more information, contact H. Stabile, University of Brescia, University of Brescia, Dept. of Biomedical Sciences and Biotechnology, Italy. Study 2: The latex protein content in devices commonly used in hospitals and general practice are investigated. "In this study the latex protein content in devices commonly used in hospitals and general practice were investigated. The main aim was to acquire information for preventing latex allergy in health care workers and in the general population. About 22 different types of medical devices and 23 devices commonly used in general practice were examined evaluating the total allergenic potency by a modified RAST-inhibition assay and quantitative determination of single allergens (Hev b1, Hev b5 and Hev b6.02) by using commercial ELISA kit," scientists writing in the journal

I International Archives of Occupational and Environmental Health report. "A high level of inhibition was found in medical devices, such as elastic bandage (81.57%), tourniquet (74.09%), Foley urinary catheter

(68.35%), Penrose drainage (67.25%) and taping (39.6%), and in common devices, such as rubber inner-sole (84.20%), toy balloon (78.62%), latex mattress (74.27%), household rubber gloves (49.10%), working gloves (38.25%), inflatable floating mattress (32.10%). Concentrations of latex extractable proteins and Hev b1, Hev b5 and Hev b6.02 antigens were high in some medical and general devices," wrote M. Crippa and colleagues, University of Brescia. They continued, "Latex exposure sources were found in hospitals and the home. These findings, though only preliminary and far from conclusive, could enable sensitized persons to avoid risky exposures and prevent allergic reactions." The researchers concluded, "From the point of view of prevention, the time may come when every natural rubber object could be systematically labelled as 'containing latex' together with the warning that 'this item may cause allergic reactions in sensitized subjects'." Crippa and colleagues published their study in the International Archives of Occupational and Environmental Health (Prevention of latex allergy among health care workers and in the general population: latex protein content in devices commonly used in hospitals and general practice. Int Arch Occup Environ Health, 2006;79(7):550-557). Additional information can be obtained by contacting M. Crippa, University of Brescia, Institute Occupational Health, Regional Hospital Sp. Civili of Brescia, Brescia, Italy. Study 3: Soluble pattern recognition receptor long pentraxin 3 (PTX3) contains an antiangiogenic binding site. According to recent research from Italy, "PTX3 is a soluble pattern recognition receptor with non-redundant functions in inflammation and innate immunity. PTX3 comprises a pentraxin-like C-terminal domain involved in complement activation via C1q interaction and an N-terminal extension with unknown functions." "PTX3 binds fibroblast growth factor-2 (FGF2), inhibiting its pro-angiogenic and pro-restenotic activity. Here, retroviral transduced endothelial cells (ECs) overexpressing the N-terminal fragment PTX3-(1-178) showed reduced mitogenic activity in response to FGF2. Accordingly, purified recombinant PTX3-(1-178) binds FGF2, prevents PTX3/FGF2 interaction, and inhibits FGF2 mitogenic activity in ECs," said M. Camozzi and colleagues, University of Brescia. "Also," researchers wrote, "the monoclonal antibody mAb-MNB4, which recognizes the PTX3-(87-99) epitope, prevents FGF2/PTX3 interaction and abolishes the FGF2 antagonist activity of PTX3. Consistently, the synthetic peptides PTX3-(82-110) and PTX3-(97-110) bind FGF2 and inhibit the interaction of FGF2 with PTX3 %%%immobilized%%% to a BIACore sensor chip, FGF2-dependent EC proliferation, and angiogenesis in vivo. "Thus, the data identify a FGF2-binding domain in the N-terminal extension of PTX3 spanning the PTX3-(97-110) region, pointing to a novel function for the N-terminal extension of PTX3 and underlining the complexity of the PTX3 molecule for modular humoral pattern recognition." Camozzi and colleagues published their study in the Journal of Biological Chemistry (Identification of an antiangiogenic FGF2-binding site in the N terminus of the soluble pattern recognition receptor PTX3. J Biol Chem, 2006;281(32):22605-22613). For additional information, contact M. Presta, University of Brescia, Dept. of Biomedical Science & Biotechnology, School Medical, Unit General Pathology & Immunology, Viale Europa 11, I-25123 Brescia, Italy. Keywords: Brescia, Italy, Biotechnology, Angiogenesis, Inflammation, Innate Immunity, Immunology, Long Pentraxin 3, Fibroblast Growth Factor, Restenosis, Recombinant Technology, Molecular Biology, Proteomics, Soluble Pattern Recognition Receptor. This article was prepared by Pharma Business Week editors from staff and other reports. Copyright 2007, Pharma Business Week via NewsRx.com & NewsRx.net.

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0000496636 (THIS IS THE FULLTEXT)  
Findings from Case Western Reserve University, U.S., advance medical research  
Pharma Business Week, April 16, 2007, p.2221

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English  
RECORD TYPE: FULLTEXT  
AUDIENCE: Professional

TEXT: Findings from Case Western Reserve University, U.S., advance medical research. This trend article about Case Western Reserve University, U.S., is an immediate alert from NewsRx to identify developing directions of research. Study 1: Research findings, "BMP signaling regulates PGC numbers and motility in organ culture," are discussed in a new report. According to a study from the United States, "Members of the bone morphogenetic protein (BMP) family play diverse roles in multiple developmental processes. However, in the mouse, mutations in many BMPs, BMP receptors and signaling components result in early embryonic lethality making it difficult to analyze the role of these factors during organogenesis or tissue homeostasis in the adult." "To bypass this early lethality, we used an organ culture system to study the role of BMPs during primordial germ cell (PGC) migration. PGCs are the embryonic precursors of the sperm and eggs. BMPs induce formation of primordial germ cells within the proximal epiblast of embryonic day 7.5 (E7.5) mouse embryos. PGCs then migrate via the gut to arrive at the developing gonads by E10.5. Addition of %%%BMP4%%% or the BMP-antagonist Noggin to transverse slices dissected from E9.5 embryos elevated PGC numbers or reduced PGC numbers, respectively. Noggin treatment also slowed and randomized PGC movements, resulting in a failure of PGCs to colonize the urogenital ridges (UGRs). Based on p-Smad1/5/8 staining, migratory PGCs do not respond to endogenous BMPs. Instead, the somatic cells of the urogenital ridges exhibit elevated p-Smad1/5/8 staining revealing active BMP signaling within the UGRs. Noggin treatment abrogated p-Smad staining within the UGRs and blocked localized expression of Kitl, a cytokine known to regulate the survival and motility of PGCs and Id1, a transcription factor expressed within the UGRs," wrote B.M. Dudley and colleagues, Case Western Reserve University, Department of Genetics. The researchers concluded: "We propose that BMP signaling regulates PGC migration by controlling gene expression within the somatic cells along the migration route and within the genital ridges." Dudley and colleagues published the results of their research in Mechanisms of Development (BMP signaling regulates PGC numbers and motility in organ culture. Mechanisms of Development, 2007;124(1):68-77). For additional information, contact B.M. Dudley, Case Western Reserve University, Dept. of Genetics, 10900 Euclid Avenue, Cleveland OH 44106 USA. Study 2:

: Mycobacterium tuberculosis phosphatidylinositol mannoside binds directly to a T cell receptor resulting in a change in CD4+ T cell function. "The pathological hallmark of the host response to Mycobacterium tuberculosis is the granuloma where T cells and macrophages interact with the extracellular matrix (ECM) to control the infection. "Recruitment and retention of T cells within inflamed tissues depend on adhesion to the ECM. T cells use integrins to adhere to the ECM, and fibronectin (FN) is one of its major components," scientists in the United States report. According to R.E. Rojas and colleagues at Case Western Reserve University in Cleveland, "We have found that the major M. tuberculosis cell wall glycolipid, phosphatidylinositol mannoside (PIM), induces homotypic adhesion of human CD4+ T cells and T cell adhesion to %%%immobilized%%% FN. "Treatment with EDTA and cytochalasin D prevented PENI-induced T cell adhesion. PIM-induced T cell adhesion to FN was blocked with mAbs against alpha(5) integrin chain and with RGD-containing peptides." alpha(5)beta(1), (VLA-5) is one of two major FN receptors on T cells. PIM was found to bind directly to purified human VLA-5. Thus, PIM interacts directly with VLA-5 on CD4+ T lymphocytes, inducing activation of the integrin, and promoting adhesion to the ECM glycoprotein, FN," researchers said. Rojas concluded, "This is the first report of direct binding of a M. tuberculosis molecule to a receptor on human T cells resulting in a change in CD4+ T cell function." Rojas and colleagues published their study in the Journal of Immunology (Phosphatidylinositol mannoside from Mycobacterium tuberculosis binds alpha(5)beta(1) integrin (VLA-5) on CD4+ T cells and induces adhesion to fibronectin. J Immunol. 2006;177(5):2959-2968). For additional information, contact R.E. Rojas, Case Western Reserve University, Dept. of Medical School Medical, 10900 Euclid Avenue, Cleveland, OH 44106, USA. Study 3: A recent study from the United States describes alterations in the oligodendrocyte lineage, myelin, and white matter in adult mice lacking the chemokine receptor CXCR2. According to the study report, "Oligodendrocyte precursor cell (OPC) proliferation and migration are critical for the development of myelin in the central nervous system (CNS). Previous studies showed that localized expression of the chemokine CXCL1 signals through the

receptor CXCR2 to inhibit the migration and enhance the proliferation of spinal cord OPCs during development. "Here, we report structural and functional alterations in the adult CNS of Cxcr2 -/- mice. In Cxcr2 -/- adult mice, we observed regional alterations in the density of oligodendrocyte lineage cells in Cxcr2 -/- adult mice, with decreases in the cortex and anterior commissure but increases in the corpus callosum and spinal cord," wrote D.A. Padovani-Claudio and colleagues, Case School of Medicine. "An increase in the density and arborization of spinal cord NG2 positive cells was also observed in Cxcr2 -/- adult mice. Compared with wild-type (WT) littermates, Cxcr2 -/- mice exhibited a significant decrease in spinal cord white matter area, reduced thickness of myelin sheaths, and a slowing in the rate of central conduction of spinally elicited evoked potentials without significant changes in axonal caliber or number," they explained. "Biochemical analyses showed decreased levels of myelin basic protein (MBP), proteolipid protein (PLP), and glial fibrillary acidic protein (GFAP). In vitro studies showed reduced numbers of differentiated oligodendrocytes in Cxcr2 -/- spinal cord cultures," the authors noted. They concluded, "Together, these findings indicate that the chemokine receptor CXCR2 is important for the development and maintenance of the oligodendrocyte lineage, myelination, and white matter in the vertebrate CNS." Padovani-Claudio and colleagues published their study in Glia (Alterations in the oligodendrocyte lineage, myelin, and white matter in adult mice lacking the chemokine receptor CXCR2. Glia, 2006;54(5):471-483). For additional information, contact R.H. Miller, Case School of Medicine, Department of Neuroscience, Cleveland, OH 44106, USA. Keywords: Cleveland, Ohio, United States, Central Nervous System, Chemokine Receptors, Conduction Velocity, Myelination, Neurology, Oligodendrocyte Precursors. This article was prepared by Pharma Business Week editors from staff and other reports. Copyright 2007, Pharma Business Week via NewsRx.com & NewsRx.net.

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1/7/4 (Item 3 from file: 135)  
DIALOG(R)File 135:NewsRx Weekly Reports  
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0000449025 (THIS IS THE FULLTEXT)  
Researchers from Lund University, Sweden, report recent findings  
Biotech Business Week, February 19, 2007, p.1042

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English  
RECORD TYPE: FULLTEXT  
AUDIENCE: Professional  
WORD COUNT: 988

TEXT: Researchers from Lund University, Sweden, report recent findings.  
This trend article about Lund University, Sweden, is an immediate alert from NewsRx to identify developing directions of research.

Study 1: A new study, "Smad5 is dispensable for adult murine hematopoiesis," is now available. "Smad5 is known to transduce intracellular signals from bone morphogenetic proteins (BMPs), which belong to the transforming growth factor-beta (TGF-beta) superfamily and are involved in the regulation of hematopoiesis. Recent findings suggest that %%%BMP4%%% stimulates proliferation of human primitive hematopoietic progenitors in vitro, while early progenitors from mice deficient in Smad5 display increased self-renewal capacity in murine embryonic hematopoiesis," scientists in Lund, Sweden report.

"Here, we evaluate the role of Smad5 in the regulation of hematopoietic stem cell (HSC) fate decisions in adult mice by using an inducible MxCre-mediated conditional knockout model. Surprisingly, analysis of induced animals revealed unperturbed cell numbers and lineage distribution in peripheral blood (PB), bone marrow (BM), and the spleen. Furthermore, phenotypic characterization of the stem cell compartment revealed normal numbers of primitive lin(-)Sca-1(+)c-Kit(+) (LSK) cells in Smad5(-)(-) BM. When transplanted in a competitive fashion into lethally irradiated primary and secondary recipients, Smad5-deficient BM cells competed normally with wild-type (wt) cells, were able to provide long-term reconstitution for the hosts, and displayed normal lineage distribution. Taken together, Smad5-deficient HSCs from adult mice show unaltered

differentiation, proliferation, and repopulating capacity," wrote S. Singbrant and colleagues, Lund University.

The researchers concluded: "Therefore, in contrast to its role in embryonic hematopoiesis, Smad5 is dispensable for hematopoiesis in the adult mouse."

Singbrant and colleagues published their study in Blood (Smad5 is dispensable for adult murine hematopoiesis. *Blood*, 2006;108(12):3707-12).

For additional information, contact S. Singbrant, Lund University, BMC A12, 221 84 Lund, Sweden.

Study 2: A study from Sweden has reported that complement activation is regulated by C-reactive protein (CRP).

"CRP is the major acute phase protein in humans. It has been shown that CRP interacts with factor H, an inhibitor of the alternative pathway of complement, and now we demonstrate binding of CRP to the fluid-phase inhibitor of the classical pathway, C4b-binding protein (C4BP)," wrote A.P. Sjoberg and colleagues, Lund University.

"C4BP bound to directly %immobilized% recombinant CRP as well as CRP attached to phosphorylcholine. The binding was sensitive to ionic strength and was enhanced in the presence of calcium. C4Bp lacking beta-chain and protein S, which is a form of C4BP increasing upon inflammation, bound CRP with higher affinity than the C4BP-protein S complex.

"The binding could not be blocked with mAbs directed against peripheral parts of the a-chains of C4BP while the isolated central core of C4BP obtained by partial proteolytic digestion bound CRP, indicating that the binding site for CRP is localized in the central core of the C4BP molecule," wrote the researchers.

"Furthermore, we found complexes in serum from a patient with an elevated CRP level and trace amounts of CRP were also identified in a plasma-derived C4BP preparation. We were also able to detect C4BP-CRP complexes in solution and established that C4BP retains full complement regulatory activity in the presence of CRP.

"In addition, we found that C4BP can compete with C1q for binding to %immobilized% CRP and that it inhibits complement activation locally," the investigators wrote.

They concluded, "We hypothesize that CRP limits excessive complement activation on targets via its interactions with both factor H and C4BP."

Sjoberg and colleagues published their study in the Journal of Immunology (Regulation of complement activation by C-reactive protein: Targeting of the inhibitory activity of C4b-binding protein. *J Immunol*, 2006;176(12):7612-7620).

For more information, contact A.M. Blom, Lund University, Department of Laboratory Medicine, Section of Clinical Chemistry, Wallenberg Laboratory, University Hospital Malmo, S-20502 Malmo, Sweden.

Study 3: Abrupt blood pressure changes during hemodialysis are detected with a proposed electrocardiogram-based method.

According to a recent report from Sweden, "Clinical techniques for early detection of acute hypotension during conventional hemodialysis treatment are lacking, even though intradialytic hypotension is the most common acute complication. In this article, intradialytic hypotension is identified by means of signal analysis of data recorded at two clinics."

K. Solem and colleagues at Lund University wrote, "The database consists of 30 treatments with concurrently acquired signals: the 12-lead electrocardiogram, continuous blood pressure, hematocrit, oxygen saturation, relative blood volume, and important hemodialysis variables."

"This article presents two characteristics, a heart rate turbulence (HRT) measure called turbulence slope (TS), and the LF/HF ratio, which provide information, at the beginning of hemodialysis treatment, on the patient's propensity to hypotension (TS: p=0.0038, and LF/HF ratio: p=0.0028)," they continued. "[We] also present a novel dynamic echocardiography-based method for detecting intradialytic hypotension using complementary information on heart rate variability (HRV) and ectopic beat patterns."

"These two types of information reflect different mechanisms of cardiac activity," the authors emphasized. "It is essential that both types are used for the detection of hypotension, because HRV analysis is inappropriate when several ectopic beats are present."

"The proposed dynamic echocardiography-based method offers early identification of the cases with acute intradialytic hypotension of the database," they stated.

Solem and colleagues published their study in the ASAIO Journal (An electrocardiogram-based method for early detection of abrupt changes in blood pressure during hemodialysis. *ASAIO J*, 2006;52(3):282-290).

For additional information, contact K. Solem, Lund University, Dept. of Electroscience, Signal Processing Group, Box 118, SE-22100 Lund, Sweden.

Keywords: Lund, Sweden, Acute, Blood Pressure, Cardiology, Cardiovascular, Database, Diagnostics, Dialysis, Echocardiography, Electrocardiogram, Heart Rate Variability, Hemodialysis, Hypotension, Intradialytic, Kidney Disease, Nephrology, Turbulence Slope.

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17/5 (Item 1 from file: 357)

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0389646 DBR Accession No.: 2006-03142 PATENT

Determining predisposition to neoplastic or cell proliferation disorder comprises determining the ratio of undifferentiated to differentiated cells in the same or different sample - investigation of pancreas cancer and pharmacogenetics involving expression profiling of a biomarker e.g. ras gene

AUTHOR: FEINBERG A P; IACOBUZIO-DONAHUE C; LONGO D L; KO M

PATENT ASSIGNEE: UNIV JOHNS HOPKINS; US NAT INST OF HEALTH 2005

PATENT NUMBER: WO 2005118878 PATENT DATE: 20051215 WPI ACCESSION NO.:

2006-056594 (200606)

PRIORITY APPLIC. NO.: US 656470 APPLIC. DATE: 20050224

NATIONAL APPLIC. NO.: WO 2005US19735 APPLIC. DATE: 20050603

LANGUAGE: English

ABSTRACT: DETERWENT ABSTRACT: NOVELTY - Determining predisposition of a

subject to developing a neoplastic or cell proliferation disorder comprises determining the ratio of undifferentiated to differentiated cells in the same or different sample from the subject, where the ratio of undifferentiated to differentiated cells, as compared to a reference ratio, is indicative of a predisposition for developing a neoplastic or cell proliferation disorder. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method of determining whether a subject is predisposed to developing a cell proliferation or neoplastic disorder; (2) a diagnostic kit, for detecting a cell proliferation or neoplastic disorder, or a predisposition to a cell proliferation or neoplastic disorder, the kit comprising an array for detecting a biomarker indicative of a differentiated or undifferentiated cells, the array comprising a substrate having addresses, each address having disposed into it an %immobilized% biomolecule, where each biomolecule individually detects a biomarker indicative of a differentiated or undifferentiated cells, and optionally instructions for using the array; (3) a method of determining whether a therapy regimen is effective for preventing or inhibiting a cell proliferation or neoplastic disorder; (4) a method of preparing an undifferentiated cell; and (5) a method of producing an altered cell population comprising undifferentiated cells capable of being recommitted into more differentiated cells. BIOTECHNOLOGY - Preferred Method: The method further comprises identifying a cell displaying abnormal expression of a target gene that directly or indirectly results from loss of imprinting. The target gene is H19 or IGF2. The target gene is Igf1R, IRS-1, IRS-2, PBK, Akt, p70S6 kinase, FOXO, GSK3, MDM2, mTOR, Cyclin D1, c-Myc, She, Grb2, SOS, Ras, Raf, MEK, Erk, or MAPK gene. The method comprises analyzing the biological sample for a change in the methylation status of a target gene, or its polymorphism. The change in methylation is hypomethylation. The method comprises analyzing the biological sample for hypomethylation of both a DMR of the H19 gene and a DMR of the IGF2 gene. The reference ratio is generated from tissue obtained from a subject comprising cells displaying normal imprinting of at least one of the H19 gene and the IGF2 gene. Determining a change in the balance or ratio of undifferentiated to differentiated cells in the sample comprises identifying a biomarker associated with a differentiated or undifferentiated cell. The biomarker is Shh (Sonic

hedgehog), Tcf4, Lef1, Twist, EphB2, EphBS, Hes1, Notch 1, Hoxa9, Dkk1, Tle6, Tcf3, Bmil, Kit, Musashil (Msil), Cdx1, Hes5, Oct4, Ki-67, beta-catenin, Noggin, %%%BMP4%%% , PTEN (phosphorylated PTEN), Akt (phosphorylated Akt), Villin, Aminopeptidase N (anpep), Sucrase isomaltase (SI), Ephrin-B1 (EfnB1), Cdx2, Grip, Apoal, Aldh1b1, Calb3, Dgat1, Dgat2, Clu, Hephaestin, Gas1, Ihh (Indian hedgehog), Intrinsic factor B12 receptor, IFABP, or KLF4. Determining the ratio of undifferentiated to differentiated cells in the sample comprises imaging the sample using immunohistochemical identification of biomarker molecules specifically associated with a differentiated or undifferentiated cell population, imaging the sample using standard microscopy and distinguishing differentiated from undifferentiated cells using morphologic measurements, imaging the sample using immunohistochemical identification of proliferation antigens and their distribution within colonic crypts, imaging the sample using immunofluorescent identification of molecules specific to a biomarker associated with a differentiated or undifferentiated cell population, measuring RNA levels, measuring gene expression, whole genome expression analyses, or allele specific expression. The cells are epithelial cells obtained from a rectal Pap test, intestinal tissue (e.g. colon or lumen of the intestinal tissue, specifically crypts of the lumen). The subject is not previously known to have a colorectal neoplasm. The method further comprises correlating the ratio derived from the subject with the subject's family genetic history. The subject is subjected to additional tests selected from chest X-rays, colorectal examinations, endoscopic examination, MRI5 CAT scanning, gallium scanning, or barium imaging. The subject is a human. Determining whether a subject is predisposed to developing a cell proliferation or neoplastic disorder comprises identifying a subject comprising cells displaying increased levels of a target gene expression, and determining the ratio of undifferentiated to differentiated cells in the same or different sample from the subject, where the ratio of undifferentiated to differentiated cells, as compared to a reference ratio, is indicative of the subjects predisposition for developing a cell proliferation or neoplastic disorder. The increased levels of the target gene expression includes increased levels of target gene mRNA and/or increased levels of a polypeptide encoded by the target gene. The increased levels of IGF2 gene expression include increased levels of IGF2 mRNA and/or IGF2 polypeptide. Alternatively, the method comprises contacting a normal biological sample from the subject with an array of %%%immobilized%%% biomolecules that specifically interact with a biomarker indicative of a differentiated or undifferentiated cell, obtaining a subject profile by detecting a modification of the biomolecules, where the modification is indicative of the ratio of differentiated to undifferentiated cells in the sample, and comparing the subject profile with a reference profile, where the reference profile comprises one or more values, each value representing the level of biomarker in a reference sample obtained from one or more reference subjects that are not predisposed to developing a cell proliferation or neoplastic disorder. The biomolecules are proteins, e.g. antibodies (monoclonal antibodies). The biomolecules are antigens or receptors. The modification is binding Shh (Sonic hedgehog), Tcf4, Lef1, Twist, EphB2, EphBS, Hes1, Notch 1, Hoxa9, Dkk1, Tle6, Tcf3, Bmil, Kit, Musashil (Msil), Cdx1, Hes5, Oct4, Ki-67, beta-catenin, Noggin, %%%BMP4%%% , PTEN (phosphorylated PTEN), Akt (phosphorylated Akt), Villin, Aminopeptidase N (anpep), Sucrase isomaltase (SI), Ephrin-B1 (EfnB1), Cdx2, Grip, Apoal, Aldh1b1, Calb3, Dgat1, Dgat2, Clu, Hephaestin, Gas1, Ihh (Indian hedgehog), Intrinsic factor B12 receptor, IFABP, or KLF4 to a biomolecule. Determining whether a therapy regimen is effective for preventing or inhibiting a cell proliferation or neoplastic disorder comprises identifying a subject at risk for developing a cell proliferation or neoplastic disorder, administering to the subject a therapy that inhibits or prevents an increase in the number of undifferentiated cells in a target tissue of the subject, contacting a biological sample comprising non-neoplastic cells from the subject with an array of %%%immobilized%%% biomolecules that specifically interact with a biomarker indicative of a differentiated or undifferentiated cell, obtaining a subject profile by detecting a modification of the biomolecules, where the modification is indicative of the ratio of differentiated to undifferentiated cells in the sample,

and comparing the subject profile with a reference profile, where the reference profile comprises one or more values, each value representing the level of biomarker in a reference sample obtained from one or more reference subjects not displaying a cell proliferation or neoplastic disorder. The method further comprises providing the determination to a caregiver and altering the therapy based upon the determination. Preparing an undifferentiated cell comprises contacting a more committed cell with an agent that causes the more committed cell to dedifferentiate into an undifferentiated cell, where the agent affects the imprinting of at least one of the H19 gene and the IGF2 gene. The committed cells are non-cancer cells or differentiated cells. Producing an altered cell population comprising undifferentiated cells capable of being recommitted into more differentiated cells comprises contacting an initial cell population comprising committed cells with an agent that modulates the imprinting status of a target gene in a cell derived from epithelial tissue, culturing the cells, and identifying the cells undifferentiated cells or recovering the undifferentiated cells from the altered cell population. Preferred Kit: The kit further comprises a means for identifying a subject comprising cells displaying abnormal expression of at least one target gene. USE - The methods and kits are useful for determining predisposition of a subject to developing a neoplastic or cell proliferation disorder, where cell proliferation or neoplastic disorder is associated with a solid tumor, e.g. an adenoma such as colorectal cancer or pancreatic cancer. EXAMPLE - No relevant example given.(79 pages)

1/7/6 (Item 2 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

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0346396 DBR Accession No.: 2004-18688 PATENT

Directing cells to differentiate along a mesodermal cell lineage, useful for tissue repair, regeneration and/or augmentation, comprises culturing the cells with bone morphogenetic protein 4 - protocol for differentiation of a stem cell into a mesodermal cell characterized by brancyury cell surface marker useful in tissue engineering application

AUTHOR: RATHJEN P D; RATHJEN J; HARVEY N T

PATENT ASSIGNEE: RATHJEN P D; RATHJEN J; HARVEY N T 2004

PATENT NUMBER: US 20040121464 PATENT DATE: 20040624 WPI ACCESSION NO.:

2004-468187 (20044)

PRIORITY APPLIC. NO.: US 675938 APPLIC. DATE: 20030930

NATIONAL APPLIC. NO.: US 675938 APPLIC. DATE: 20030930

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Directing a population of cells to differentiate along a mesodermal cell lineage comprising culturing the cells in the presence of bone morphogenetic protein 4 (%%%BMP4%%% ) or its homologue, analogue or functional equivalent, for a time and under conditions for the cells to preferentially differentiate into mesodermal cells or cells of a mesodermal lineage, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) generating mesodermal cells from embryonic stem (ES) or early primitive ectoderm-like (EPL) cells; (2) mesodermal cells prepared by the process of (1); (3) screening for a change in a developmental stage of an EPL or other stem cell or mesodermal cell; (4) determining a developmental stage of an EPL or other stem cell or mesodermal cell or cell developmentally in-between after exposure to a potential proliferating- or differentiating- or self-renewal-stimulating agent; (5) tissue repair, regeneration and/or augmentation; and (6) a composition comprising a modulator of mesodermal cell generation from EPL or other stem cells or maintaining or expanding mesodermal cells, the composition further comprising one or more pharmaceutical carriers and/or diluents BIOTECHNOLOGY - Preferred Method: The cells are EPL cells or stem cells. The stem cells are embryonic, somatic, germ, epidermal, adult neural, keratinocyte, melanocyte, adult renal, embryonic renal epithelial, embryonic endodermal, hepatocyte, mammary epithelial, bone marrow-derived, skeletal muscle, bone marrow mesenchymal, CD34+ hematopoietic, or mesenchymal stem cells. The %%%BMP4%%% is derived from a homologous or heterologous species to the cells. The cells are isolated from an animal, e.g. primates, livestock

animals, laboratory test animals, companion animals, or avian species. The cells are isolated from a mammal, i.e. human. Generating mesodermal cells from ES or EPL cells comprises culturing ES cells or EPL cells in conditioned medium from HepG2 cells (MEDII) or its functional equivalent to generate embryoid bodies (EBM), maintaining the EBMs in culture for a time to allow aggregation of the EBMs, transferring the aggregated EBMs to gelatin-treated wells, allowing the aggregated EBMs to adhere to the gelatin-treated wells, and culturing the adhered EBMs in serum free medium comprising bone morphogenetic protein 4 (BMP4) for a time to allow the EBMs to generate mesodermal cells. Screening for a change in a developmental stage of an EPL or other stem cell or mesodermal cell comprises exposing an *in vitro* or *ex vivo* culture or suspension of EPL or other stem cell or mesodermal cells to an agent having a potential to induce proliferation and/or differentiation and/or self-renewal, where the level of proliferation and/or differentiation and/or self-renewal is determinable by a surface marker on the cells, contacting the cell surface with a ligand for the surface marker, and detecting the presence of binding to the surface marker, where the pattern of surface markers determines whether an agent has induced proliferation and/or differentiation of the EPL or other stem cell. The surface marker is brancyury. Determining a developmental stage of an EPL or other stem cell or mesodermal cell or cell developmentally in-between after exposure to a potential proliferating- or differentiating- or self-renewal-stimulating agent comprises capturing the EPL or other stem cell or mesodermal cell or cell developmentally in-between by %immobilization% to an anchored antibody to a solid support, and screening the %immobilized% cell with a range of antibodies labeled with separate reporter molecules or a range of anti-immunoglobulin antibodies each labeled with a reporter molecule used to determine existence of particular antigens, where the antigens being indicative of the developmental stage of the cell. A method for tissue repair, regeneration and/or augmentation comprises generating mesodermal cells by culturing EPL cells or stem cells in the presence of an amount of %BMP4% or its functional equivalent, for a time and under conditions to generate mesodermal cells, and introducing the mesodermal cells into a subject. The method further comprises proliferating and/or further differentiating the mesodermal cells. The tissue consists of cells of hemopoietic lineage, cells of muscle lineage, bone, connective tissue, organ tissue, or cells of the immune system. The organ tissue is heart, liver, pancreas, kidney, brain, epidermis, skin, breast, lung, head, thymus, eye, epithelium, gut, biliary system, or spleen.

**ACTIVITY - Vulnerary.** No biological data given. **MECHANISM OF ACTION -** None given. **USE -** The method is useful for directing a population of cells to differentiate along a mesodermal cell lineage. The methods and composition are useful for tissue repair, regeneration and/or augmentation. (19 pages)

1/7/7 (Item 3 from file: 357)  
DIALOG(R)File 357:Derwent Biotech Res.  
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0316052 DBR Accession No.: 2003-17192 PATENT  
Transferring nucleic acid into cells associated with fluid space by contacting wound site situated in tissue associated with fluid space, with composition comprising nucleic acid and biocompatible matrix - gene transfer expression in cell for use in disease therapy and gene therapy

AUTHOR: SOSNOWSKI B A; PIERCE G

PATENT ASSIGNEE: SELECTIVE GENETICS INC 2003

PATENT NUMBER: WO 200329429 PATENT DATE: 20030410 WPI ACCESSION NO.:

2003-430202 (200340)

PRIORITY APPLIC. NO.: US 327513 APPLIC. DATE: 20011003

NATIONAL APPLIC. NO.: WO 2002US31546 APPLIC. DATE: 20021002

LANGUAGE: English

**ABSTRACT:** DERWENT ABSTRACT: NOVELTY - Transferring (M1) a nucleic acid molecule into cells associated with a fluid space, involves contacting a wound site with a composition (I) comprising a nucleic acid molecule and a biocompatible matrix, the wound site being situated in a tissue

associated with the fluid space. **DETAILED DESCRIPTION - INDEPENDENT CLAIMS** are also included for: (1) stimulating (M2) gene expression in cartilage progenitor cells located within a cartilage progenitor tissue site of an animal, involves contacting the tissue site with a composition comprising a chondrogenic gene and a biocompatible matrix; (2) stimulating (M3) cartilage repair or regeneration, by implanting at a cartilage defective site a matrix-gene composition comprising a chondrogenic gene and a biocompatible matrix; (3) treating (M4) arthritis, by implanting at a cartilage defective site a matrix-gene composition comprising chondrogenic gene and a biocompatible matrix; (4) treating (M5) ischemic heart disease by implanting a matrix-gene composition comprising an angiogenic gene and a biocompatible matrix into an ischemic region; and (5) a composition comprising multiple genes associated with a multi-partitional biocompatible matrix. **BIOTECHNOLOGY - Preferred Method:** The wound site is situated in a tissue e.g., cartilage, cardiac muscle or bone/cartilage interface, associated with the fluid space. The method involves contacting (I) with a wound site which is a wound induced by injury or a disease state, or an iatrogenic wound. The contacting process involves bringing the nucleic acid molecule into contact with the biocompatible matrix to form a matrix-nucleic acid composition and bringing the matrix-nucleic acid composition into contact with the tissue site. The nucleic acid molecule is a DNA molecule complexed with anti-DNA antibodies, histone H1, a polycation or is a DNA molecule comprising a promoter operably linked to a sequence encoding a gene product. The DNA molecule encodes a therapeutic protein such as a growth factor chosen from transforming growth factor (TGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF), bone morphogenic factor (BMP) or a cartilage-derived morphogenic protein (CDMP). Optionally, the therapeutic protein is a growth hormone or human parathyroid hormone (PTH). The therapeutic protein may be latent TGF-beta binding protein (LTBP), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), Factor VIII, Factor IX, erythropoietin (EPO), tissue plasminogen activator (TPA), leukemia inhibitory factor (LIF), parathyroid hormone-related peptide (PTHRP), activin, inhibin, interleukin, macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), skeletal growth factor (SGF), chondromodulin, mono or polyclonal antibodies and its fragments, enzymes involved in production and/or processing of collagen, enzymes involved in production and/or processing of hyaluronic acid, transcription factors that trigger proliferation, differentiation, and morphogenic pathways, cell survival factors, or cell death factors. Optionally, the nucleic acid molecule is an RNA molecule, antisense nucleic acid molecule, a linear nucleic acid molecule, a plasmid or a recombinant insert within the genome of a recombinant virus. The biocompatible matrix is a biological matrix which comprises a polymer, and is chosen from collagen, purified proteins, purified peptides, polysaccharides (e.g. chitosan, alginate, dextran, hyaluronic acid and cellulose), and extracellular matrix compositions. Preferably, the biological matrix comprises type I collagen, type II collagen, mineralized collagen or atelocollagen collagen. Optionally, the biocompatible matrix is a synthetic matrix which comprises a polymer chosen from polyethylene glycols or their derivatives, polyesters, polyethers, polyanhydrides, polyalkylcyanoacrylates, polyacrylamides, polyorthoesters, polyphosphazenes, polyvinylacetates, block copolymers, polytetrafluoroethylene (PTFE), and polyurethanes. Optionally, the polymer comprises lactic acid or glycolic acid, or may be a copolymer which comprises lactic acid and glycolic acid (PLGA). The biocompatible matrix is biodegradable or non-biodegradable. The non-biodegradable matrix comprises a polymer such as poly(dimethylsiloxane) or poly(ethylene-vinyl acetate). The biocompatible matrix is a collagen, metal, hydroxyapatite, bioglass, aluminate, bioceramic materials, hyaluronic acid polymers, acrylic ester polymer, lactic acid polymer, glycolic acid polymer, lactic acid/glycolic acid polymer, purified proteins, purified peptides, and extracellular matrix compositions. **Preferred Method:** In (M2), the contacting process involves bringing the chondrogenic gene with the biocompatible matrix to form a matrix-gene composition and bringing the matrix-gene composition into contact with

the tissue site. The biocompatible matrix is a collagen preparation, hydroxyapatite matrix, a lactic acid polymer matrix or a fibrin matrix. In (M3), the matrix comprises a first portion and a second portion. The first portion comprises a gene to stimulate cartilage growth and the second portion comprises a gene to stimulate bone growth. ACTIVITY - Vulnerary; Antiarthritic; Antiinflammatory; Vasotropic. MECHANISM OF ACTION - Gene therapy. Influence of collagen-%%%immobilized%%% fibroblast growth factor (FGF) genes on muscle wound repair was examined using the rodent hind limb model. At day 14 following delivery of DNA(FGF2) formulated in a blend of 1% collagen and 1% gelatin, trichrome stains revealed that these matrices were well infiltrated by both mononuclear cells and elongated fibroblastoid cells. Many of these cells were organized around simple single-walled vessel, and may represent vascular precursors giving rise to neovasculature. The presence of erythrocytes with vessel lumens confirmed that these vessels were perfused. By day 21 post-treatment, in addition to microvasculature, well-organized muscular arterioles were also present. Skeletal muscle bundles were scattered throughout the collagen-gelatin matrix, which appeared to be reduced in volume over that seen at day 14. neither the residual matrix nor the surrounding tissue showed any signs of edema. Very similar observations were seen following the delivery of collagen-gelatin-DNA(FGF6) to muscle wounds, including the development of both micro- and macrovasculature. Delivery of the control transgene luciferase induced a much different response. Even at day 21, considerable collagen-gelatin matrix remained, and although a mononuclear cell infiltrate was present, blood- perfused vessels perfused were rare. infiltrating cells were organized into discrete areas, however the majority of these structures were not true vasculature in that they were not lined by a continuous endothelium and were not perfused with blood. Finally, delivery of FGF2 protein was seen to induce a limited angiogenic response comprised of small capillaries. Arteriogenesis similar to that induced by FGF2 or FGF6 gene delivery was not observed. USE - (M1) is useful for transferring a nucleic acid molecule into cells associated with a fluid space. (M2) is useful for stimulating gene expression in cartilage progenitor cells located within a cartilage progenitor tissue site of an animal, where expression of the gene in the cell stimulates the cells to promote cartilage tissue repair or regeneration. The cartilage progenitor tissue site of an animal is a site of cartilage injury (a partial-thickness injury or a full-thickness injury), or is a cartilage cavity site, or is the result of surgery or the removal of cartilage tumor. The chondrogenic gene is in the form of plasmid DNA, a DNA insert within the genome of a recombinant adenovirus, a DNA insert within the genome of a recombinant adeno-associated virus (AAV) or a DNA insert within the genome of a recombinant retrovirus. The chondrogenic gene is parathyroid hormone (PTH) gene, bone morphogenic factor (BMP) gene, a cartilage-derived morphogenic protein (CDMP) gene, a growth factor gene, a growth factor receptor gene (e.g., IGF receptor gene or MBP receptor gene), where the growth factor gene is fibroblast growth factor (FGF) gene, insulin-like growth factor (IGF) gene, hepatocyte growth factor (HGF) gene, a gene in the transforming growth factor (TGF) family of genes, epidermal growth factor (EGF) gene, connective tissue growth factor (CTGF) gene, leukemia inhibitory factor (LIF) gene, parathyroid hormone-related peptide (PTHR) gene, platelet-derived growth factor (PDGF) gene, skeletal growth factor (SGF) gene, BIP gene, MP52 gene, chondromodulin gene, preferably basic FGF gene, IGF-I or IGF-II gene, TGFalpha, TGFbeta1 or TGFbeta2, BMP2, BMP3, %%BMP4%%, BMP5, BMP6, BMP7, BMP8, BMP9, BMP10, BMP11, BMP12 or BMP13 gene. (M3) is useful for stimulating cartilage repair or regeneration. (M4) is useful for treating arthritis, where the chondrogenic gene that is implanted is an IL-4 gene, or a gene that encodes either a ribozyme that cleaves mRNAs for an inflammation mediator, or an antisense nucleic acid that binds to mRNA for an inflammation mediator such as IL-1, IL-6, IL-8, TNF-alpha, granulocyte-macrophage colony stimulating factor (GM-CSF), a soluble receptor that binds to a mediator of inflammation, or an antibody or its fragment that binds to a mediator of inflammation. (M5) is useful for treating ischemic heart disease, where the angiogenic gene that is implanted is FGF gene, VEGF gene, TNF-alpha gene, HGF gene, or a PDGF gene (all claimed). ADMINISTRATION - The gene-matrix composition is

transferred directly to the site of a naturally occurring wound or an iatrogenic injury or the matrices may be surgically placed in a wound made in an organ. The matrices may also be implanted via grafting, injection, catheterization, laproscopic surgical procedures, or arthroscopic surgery. ADVANTAGE - Direct plasmid DNA transfer from a matrix to a mammalian repair cell, through stimulation of the wound healing process, has the following advantages: (a) each are capable of producing and purifying DNA constructs; (b) matrices can act as structural scaffolds that, in and of themselves, promote cell in growth and proliferation, thus facilitating the targeting of repair cells for gene transfer; (c) the introduction of a biocompatible matrix to tissues associated with a fluid space results in less damage to surrounding tissues during introduction; (d) the biocompatible matrix may be implanted through or across the fluid space without harming other tissue; (e) the method therefore, is a minimally invasive means of utilizing gene therapy to introduce therapeutic molecules to tissues associated with fluid spaces; (f) the proximity of a fluid space facilitates the migration of repair cells to the biocompatible matrix that is inserted into a tissue associated with a fluid space; and (g) the methods are efficient in introducing gene therapy products to target cells associated with a fluid space.(95 pages)

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0306427 DBR Accession No.: 2003-08212 PATENT  
Regulating LRP5, LRP6 or HBM activity in a subject, useful for modulating lipid levels and/or bone mass, and for in treating bone mass disorders, e.g. osteoporosis, comprises administering a composition which modulates a Dkk activity - aptamer, antisense and reporter molecular for disease diagnosis and therapy  
AUTHOR: ALLEN K; ANISOWICZ A; BHAT B M; DAMAGNEZ V; ROBINSON J A; YAWORSKY P J  
PATENT ASSIGNEE: GENOME THERAPEUTICS CORP; WYETH 2002  
PATENT NUMBER: WO 200292015 PATENT DATE: 20021121 WPI ACCESSION NO.: 2003-129219 (200312)  
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NATIONAL APPLIC. NO.: WO 2002US15982 APPLIC. DATE: 20020517  
LANGUAGE: English  
ABSTRACT: DERVENT ABSTRACT: NOVELTY - Regulating LRP5, LRP6 or HBM activity in a subject comprising administering a composition which modulates a Dkk activity, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) regulating Dkk-Wnt pathway activity in a subject; (2) modulating bone mass in a subject; (3) modulating lipid levels in a subject; (4) diagnosing low or high bone mass and/or high or low lipid levels in a subject; (5) screening for a compound which modulates the interaction of Dkk with LRP5, LRP6, HBM or a Dkk-binding fragment of LRP5, LRP6 or HBM; (6) screening a compound which modulates the interaction of Dkk with a Dkk interacting protein; (7) a composition comprising an LRP5, LRP6 or HBM activity-modulating compound, and a pharmaceutical carrier; (8) a pharmaceutical composition a compound which modulates Dkk and LRP5/LRP6/HBM interactions; (9) identifying binding partners for a Dkk protein or compounds which modulate Dkk and/or LRP5/LRP6/HBM interactions; (10) a nucleic acid encoding a Dkk interacting protein peptide aptamer comprising a nucleic acid encoding a scaffold protein in-frame with the activation domain of Gal4 or Lex A that is in frame with nucleic acid that encodes a Dkk interacting protein amino acid sequence; (11) a vector comprising the nucleic acid of (10); (12) detecting a modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide-binding pair that binds through extracellular interaction in their natural environment; (13) a transgenic animal where Dkk-1 is knocked out in a tissue-specific fashion; (14) identifying potential compounds which modulate Dkk activity; (15) a peptide aptamer comprising one of 22 13-32 residue amino acid sequences, given in the specification; (16) an antibody or antibody fragment which recognizes and binds to one or more of 18 13-17 residue amino acid sequences, given in the specification; (17)

identifying Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway; (18) identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions; (19) identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway; (20) testing compounds that modulate Dkk-mediated activity in a mammal; (21) screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein; and (22) an antibody or antibody fragment which recognizes and binds to a sequence selected from 18 peptide sequences given in the specification. BIOTECHNOLOGY - Preferred Method: Dkk is Dkk-1, and Dkk activity is inhibited. The Dkk activity modulates bone mass and/or lipid levels, where bone mass is increased and/or lipid levels are decreased. Increase in bone mass is determined by a decrease in fracture rate, or by an increase in bone strength, bone density, trabecular connectivity, trabecular density, cortical density, bone diameter or inorganic bone content. The composition comprises one or more compounds selected from Dkk interacting proteins or its Dkk-binding fragment. The composition comprises an antisense, siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins. The composition may also comprise a mimetic of a Dkk peptide aptamer, a mimetic of a Dkk interacting protein peptide aptamer, a mimetic of a Dkk interacting protein peptide aptamer, or an LRP5 peptide aptamer. The composition inhibits or enhances Dkk binding to LRP5, LRP6 or HBM, or may also inhibit or enhance Dkk interacting protein or Dkk-binding fragment binding to Dkk. The peptide aptamer OST262 comprises a 154 residue amino acid sequence, given in the specification. The composition may alternatively comprise an LRP5 antibody or its immunologically active fragment. The subject is a vertebrate or an invertebrate, preferably a mammal selected from a canine, feline, ovine, primate, equine, porcine, caprine, camelid, avian, bovine and rodent, where the primate is preferably a human. Regulating Dkk-Wnt pathway activity in a subject comprises administering a composition which modulates Dkk activity, where Wnt is selected from Wnt1-Wnt19, preferably Wnt1, Wnt3, Wnt3a or Wnt10b. The composition which modulates Dkk activity or Dkk interaction with LRP5/LRP6/HBM is administered to modulate Wnt signaling. Modulating bone mass or lipid levels in a subject comprises administering a composition which modulates Dkk activity or Dkk interaction with LRP5, LRP6 or HBM, where bone mass is increased. Increase in bone mass is determined by a decrease in fracture rate, or by an increase in bone strength, bone density, bone mineral density, trabecular connectivity, trabecular density, cortical density, bone diameter or inorganic bone content. The subject has a bone mass disorder such as bone development disorder, bone fracture, age-related loss of bone, chondrodystrophy, drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteoporosis, Paget's disease, osteoarthritis, or rickets. The composition is administered to modulate the amount of trabecular and/or cortical tissue. The lipid-modulated disorder is a cardiac condition, atherosclerosis, familial lipoprotein lipase deficiency, familial apoprotein CII deficiency, familial hypertriglyceridemia, multiple lipoprotein-type hyperlipidemia, elevated lipid levels due to dialysis and/or diabetes, or elevated lipid levels of unknown etiology. Diagnosing low or high bone mass and/or high or low lipid levels in a subject comprises examining expression of Dkk, LRP5, LRP6, HBM and/or HBM-like variant in the subject, and determining whether these are over- or under-expressed. Screening for a compound which modulates the interaction of Dkk with LRP5, LRP6, HBM or a Dkk-binding fragment of LRP5, LRP6 or HBM, comprises exposing Dkk and an LRP5, LRP6 and/or HBM binding fragment to a compound, and determining whether the compound modulates Dkk interaction with the LRP5, LRP6 and/or HBM binding fragment, where modulation is determined by determining if the compound binds to Dkk or the LRP5, LRP6 and/or HBM binding fragment. The Dkk or an LRP-binding fragment is attached to a substrate. The compound comprises one or more Dkk interacting proteins or Dkk binding fragment, a Dkk peptide aptamer, a mimetic of a Dkk peptide aptamer, a Dkk interacting protein peptide aptamer, an LRP5 peptide aptamer, an LRP5 antibody, or a mimetic of a Dkk interacting protein peptide aptamer. Screening a compound which modulates the interaction of Dkk with a Dkk interacting protein comprises exposing a Dkk interacting protein or a Dkk-binding

fragment to a compound, determining whether the compound binds to the Dkk interacting protein or Dkk-binding fragment, and further determining whether the compound modulates the interaction of Dkk interacting protein and Dkk. Identifying compounds, which modulate Dkk and/or LRP5/LRP6/HBM interactions, comprises creating an LRP5, LRP6 or HBM fluorescent fusion protein using a fluorescent tag, creating a Dkk fusion protein comprising a second fluorescent tag, adding a test compound, and assessing changes in the ratio of fluorescent tag emissions using fluorescence Resonance energy transfer (FRET) or bioluminescence resonance Energy Transfer (BRET) to determine whether the compound modulates Dkk and LRP5/LRP6/HBM interactions. The method may alternatively comprise %%immobilizing%% LRP5/LRP6/HBM to a solid surface, treating the solid surface with a secreted Dkk protein or epitope-tagged Dkk and a test compound, and determining whether the compound regulates binding between Dkk and LRP5/LRP6/HBM using antibodies to Dkk or the epitope tag, or by directly measuring the activity of an epitope tag. The epitope tag is alkaline phosphatase, histidine, or a V5 tag. Identifying binding partners for a Dkk protein comprises exposing the Dkk protein or LRP5/LRP6 binding fragment to a potential binding partner, and determining if the potential binding partner binds to a Dkk protein or the LRP5/LRP6 binding fragment. Detecting a modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide-binding pair that binds through extracellular interaction in their natural environment, comprises: (a) culturing at least one eukaryotic cell comprising a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or its segment joined to a transcriptional activation protein DNA binding domain, a nucleotide sequence encoding a second heterologous fusion protein comprising a second peptide or its segment joined to a transcriptional activation protein transcriptional activation domain, where binding of the first and second peptides reconstitutes a transcriptional activation protein, and a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, where expression of the reporter element produces a selected phenotype; (b) incubating the eukaryotic cell in the presence of a compound to detect the selected phenotype; and (c) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining if the compound affects the expression of the reporter element which produces the selected phenotype. The first peptide is a Dkk peptide, and the second peptide is LRP5, HBM, LRP6 or Dkk-binding portion of LRP5/LRP6/HBM. Alternatively, the first peptide is a Dkk interacting protein or Dkk-binding fragment, and the second peptide is a Dkk peptide. The eukaryotic cell is a yeast cell such as *Saccharomyces*, preferably *Saccharomyces cerevisiae*. The Dkk is Dkk-1, and the compound comprises one or more Dkk interacting proteins or a Dkk-binding fragment. The compound is directly added to the assay or is recombinantly expressed by the eukaryotic cell in addition to the first and second peptides. The eukaryotic cell further comprises at least one endogenous nucleotide sequence encoding the DNA binding domain of a transcriptional activation protein, the transcriptional activation domain of a transcriptional activation protein or the reporter element, where at least one of the endogenous nucleotide sequences is inactivated by mutation or deletion. The peptide binding pair comprises a ligand and a receptor to which the ligand binds. The transcriptional activation protein is Gal4, Gnc4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16 or a mammalian nuclear receptor. Preferably at least one of the heterologous fusion proteins is expressed from an autonomously-replicating plasmid. The DNA binding domain is a heterologous DNA-binding domain of a transcriptional activation protein, and the DNA binding protein is a mammalian steroid receptor or bacterial LexA protein. The reporter element is a LacZ, a polynucleotide encoding luciferase, a polynucleotide encoding green fluorescent protein, or a polynucleotide encoding chloramphenicol acetyltransferase, preferably LacZ. The test sample comprises an LRP5 peptide aptamer, preferably OST262, or an LRP5 antibody. Identifying potential compounds which modulate Dkk activity comprises measuring the effect on binding of one or more Dkk interacting proteins or a Dkk-binding fragment, with a Dkk or its fragment in the presence or

absence of a compound, and identifying as a potential Dkk modulatory compound a compound which modulates the binding between one or more Dkk interacting proteins or Dkk-binding fragment, and Dkk or its fragment. Identifying Dkk interacting proteins, which modulate the interaction of Dkk with the Wnt signaling pathway, comprises injecting Dkk and potential Dkk interacting protein mRNA into a Xenopus blastomere, assessing axis duplication or marker gene expression, and identifying compositions which elicit changes in axis duplication or marker gene expression as Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway. The mRNA of HBM, LRP5/6, any wnt, Wnt antagonist, Wnt pathway modulator, or a combination of these is co-injected into the Xenopus blastomere. The marker gene analyzed is Siamois, Xnr3, slug, Xbra, HNK-1, endodermin, Xlhxbox8, BMP2, %%%BMP4%%% , XLRP6, EF-1 or ODC. The method alternatively comprises transfecting cells with constructs containing Dkk and potential Dkk interacting proteins, assessing changes in expression of a reporter gene linked to a Wnt-responsive promoter, and identifying as a Dkk interacting protein in any protein which alters reporter gene expression compared with cells transfected with a Dkk construct alone. The cells are HOB-03-CE6, HKE293 or U2OS cells. The Wnt-responsive promoter is TCF or LEF. The cells are co-transfected with cytomegalovirus (CMV) beta-galactosidase. Identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway comprises transfecting cells with constructs containing Dkk and Wnt proteins, assessing changes in expression of a reporter element linked to a Wnt-responsive promoter, and identifying as Dkk/Wnt interaction modulating compound any compound which alters reporter gene expression compared to cells transfected with a Dkk construct alone. Wnt3a and Wnt1 constructs are co-transfected into the cells, where the cells are HOB-03-CE6, HKE293 or U2OS cells. The reporter element is TCF-luciferase and/or tk-Renilla. Testing compounds that modulate Dkk-mediated activity in a mammal comprises providing a group of transgenic animals having a regulatable one or more Dkk genes, a knock-out of Dkk genes or a knock in of one or more Dkk genes, providing a second group of control animals respectively for the group of transgenic animals, exposing the animals to a potential Dkk-modulating compound which modulates bone mass or lipid levels, and comparing the transgenic animals and the control group of animals and determining the effect of the compound on bone mass or lipid levels in the transgenic animals compared to the control animals. Screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprises exposing a Dkk interacting proteins or a Dkk-binding fragment to a compound, and determining whether the compound binds to a Dkk interacting proteins or Dkk-binding fragment. Modulation is determined if the compound binds to the Dkk interacting protein or the Dkk binding fragment. Preferred Composition: The composition of (7) comprises an LRP5, LRP6 or HBM activity-modulating compound that binds to Dkk thus modulating the interaction of Dkk with LRP5, LRP6 or HBM. The LRP5-, LRP6- or HBM-modulating compound comprises one or more Dkk interacting proteins and Dkk-binding fragments, a monoclonal antibody or its immunologically active fragment that binds to a Dkk interacting protein or Dkk binding fragment, an antisense, a siRNA or shRNA molecule that recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins, a Dkk peptide aptamer, a Dkk interacting protein peptide aptamer or its mimetic, an LRP5 peptide aptamer, preferably OST262, or an LRP5 antibody. ACTIVITY - Osteopathic; Antiinflammatory; Antiarthritic. No biological data is given. MECHANISM OF ACTION - Dkk modulator. USE - The method is useful for modulating lipid levels and/or bone mass, and is useful in treating or diagnosing abnormal lipid levels and bone mass disorders, such as osteoporosis, bond fracture, age-related loss of bone, a chondrodystrophy, drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis, imperfecta, osteomalacia, osteomyelitis, Paget's disease, osteoarthritis, and rickets. Modulators of Dkk activity are useful for as reagents in studying bone mass and lipid level modulation, in modulating Wnt signaling, or treating Dkk-mediated disorders. ADMINISTRATION - Dosage is 0.0001-50, preferably 0.1-1 mg/kg. Administration can be parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal. EXAMPLE - No relevant examples are given. (173 pages)

1/7/9 (Item 1 from file: 399)  
 DIALOG(R)File 399:CA SEARCH(R)  
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150032162 CA: 150(3)32162m PATENT  
 Megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte  
 INVENTOR(AUTHOR): Elefanty, Andrew; Stanley, Eduoard; Ng, Elizabeth  
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 ASSIGNEE: Australian Stem Cell Centre Ltd.  
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 SECTION:

CA213003 Mammalian Biochemistry

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IDENTIFIERS: embryonic stem cell differentiation megakaryocyte formation, BMP4 VEGF SCF FGF2 hematopoiesis megakaryocyte

DESCRIPTORS:

Thrombocytopenia...

alloimmune, Neonatal, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Platelet-derived growth factor receptors...

.alpha., expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Integrins...

.alpha.IIb, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Disease,animal...

assocd. with reduced platelet nos., megakaryocyte and megakaryocyte precursors for treating; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Integrins...

.beta.3, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Cartilage... Materials...

bioreactor matrix for differentiation of embryonic stem cells made of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Bone morphogenetic protein 4...

BMP4, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal...

Brachury, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Antigens...

CD110, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Thrombocytopenia...

Chemotherapy-induced, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Thrombocytopenia...

Congenital Amegakaryocytic, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Bone...

demineralized , bioreactor matrix for differentiation of embryonic stem cells made of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Blood coagulation disorders... disseminated intravascular coagulation, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Stem cell... embryonic; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Human... ESC from; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

CD34(antigen)... CD45(antigen)... Sialic acid-binding Ig-like lectin 3... expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Flow cytometry... FACS (fluorescence-activated cell sorting), for isolation of megakaryocyte; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Liver,disease... failure, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Anemia... Fanconi anemia, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Antibodies and Immunoglobulins... for detecting cell surface markers; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Bioreactors... for differentiation of embryonic stem cells; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... GATA1, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... GATA2, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... GOOSECOID, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Platelet disorders... Gray platelet syndrome, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Kidney,disease... hemolytic-uremic syndrome, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Purpura(disease)... idiopathic thrombocytopenic, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Interleukin 11... IL11, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Interleukin 3... IL3, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Interleukin 6... IL6, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Interleukin 9... IL9, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Cytokines... Growth factors,animal... in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Biomarkers... Cell differentiation... Cell proliferation... Hematopoiesis ... Megakaryocyte... megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

uses of megakaryocyte

Embryo,animal... mesendoderm, formation of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Embryo,animal... mesoderm, formation of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... MIKL1, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... MIXL1, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... MPL, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Immobilization,molecular or cellular... of the growth factor and cytokine on the internal surface within the bioreactor; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... PF4, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Purpura(disease)... posttransfusion purpura, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Blood platelet... prodn. of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... RUNX1, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Stem cell factor... SCF, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Gene,animal... SCL, expression of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Culture media... serum-free, stromal/feeder cell-free; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Embryo,animal... stem cell; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Lupus erythematosus... Viral infection...

Systemic , treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Bone,disease... Platelet disorders... thrombocytopenia-absent radius syndrome, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Purpura(disease)... thrombotic thrombocytopenic, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

Alport syndrome... Antiphospholipid syndrome... Bacterial infection...

Leukemia... Myelodysplastic syndromes... Paroxysmal nocturnal hemoglobinuria... Sepsis... treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

CAS REGISTRY NUMBERS:

59-30-3 biological studies, deficiency, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

68-19-9 deficiency, treatment of; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

11096-26-7 EPO, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

106096-93-9 FGF2, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

9014-42-0 TPO, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte  
127464-60-2 VEGF, in cell culture; megakaryocyte differentiation from embryonic stem cells and therapeutic uses of megakaryocyte

1/7/10 (Item 2 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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141067861 CA: 141(5)67861e PATENT  
Preparation of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in the presence of BMP4 protein  
INVENTOR(AUTHOR): Rathjen, Peter David; Rathjen, Joy; Harvey, Nathan Tobias

LOCATION: Australia

PATENT: U.S. Pat. Appl. Publ. ; US 20040121464 A1 DATE: 20040624  
APPLICATION: US 675938 (20030930) \*US PV414959 (20020930)  
PAGES: 19 pp. CODEN: USXXCO LANGUAGE: English

PATENT CLASSIFICATIONS:

CLASS: 435455000; C12N-005/08A; C12N-015/85B

SECTION:

CA209011 Biochemical Methods

CA213XXX Mammalian Biochemistry

CA263XXX Pharmaceuticals

IDENTIFIERS: stem cell culture differentiation mesoderm BMP4 tissue repair

DESCRIPTORS:

Phosphorylation,biological...

BMP4-induced phosphorylation of Smad5; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Hematopoietic precursor cell...

CD34+; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Immobilization,molecular or cellular... Antigens...

detrn. of cell developmental stage; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal...

ectoderm, EPM (early primitive ectoderm-like cells); prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal...

embryoid body; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal...

entoderm, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Skin...

epidermis, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Kidney...

epithelium, embryonic, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Mammary gland...

epithelium, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Proteins...

gene Brachyury, marker; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Liver...

hepatocyte, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Skin...

keratinocyte, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Antibodies and Immunoglobulins...

labeled, detn. of cell developmental stage; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Epithelium...

mammary, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Culture media...

MEDII; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal...

mesoderm; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Heart...

myocyte, formation of; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Nerve...

neuron, formation of; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Cell differentiation... Animal tissue culture... Stem cell... Primates...

Livestock... Laboratory animal... Pet animal... Aves... Mammalia... Human... Biomarkers(biological responses)... Wound healing...

Regeneration,animal...

prep. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Epithelium...

renal, embryonic, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Transcription factors...

Smad-5, BMP4-induced phosphorylation of; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Animal cell...

somatic, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal... Gamete and Germ cell... Nerve... Melanocyte... Kidney... Bone marrow... Muscle...

stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Hematopoietic system... Bone... Connective tissue... Organ,animal... Immune system... Heart... Liver... Pancreas... Brain... Skin... Mammary gland...

Lung... Head... Thymus gland... Eye... Epithelium... Digestive tract...

Biliary tract... Spleen...

tissue repair; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Embryo,animal... Nerve... Kidney... Bone marrow...

toxicity, stem cells; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Heart... Liver... Lung...

toxicity, tissue repair; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Gelatins,uses...

wells treated by; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

Bone morphogenetic proteins...

4; prepn. of cells of mesodermal lineage for tissue repair or regeneration by culturing stem cells in presence of BMP4 protein

? by

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\$2.57 0.090 DialUnits File34  
\$8.28 1 Type(s) in Format 7  
\$8.28 1 Types  
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\$14.34 0.524 DialUnits File357  
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